GENETIC STUDIES OF THE WEST INDIAN MANATEE
(*Trichechus manatus*)

By

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GENETIC STUDIES OF THE WEST INDIAN MANATEE
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By

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Chair: Brian W. Bowen
Major Department: Fisheries and Aquatic Sciences

Mitochondrial DNA (mtDNA) control region sequences compared among locations across the western Atlantic region showed population genetic structure and phylogeography for the West Indian manatee (*Trichechus manatus*). Haplotypes identified among individuals from Florida, Puerto Rico, the Dominican Republic, Mexico, Colombia, Venezuela, Guyana, and Brazil indicated strong population separations between most locations. Three distinctive mtDNA lineages were observed in an intraspecific phylogeny of *T. manatus*: (1) Florida and the West Indies, (2) Gulf of Mexico and Caribbean rivers of South America, and (3) northeast Atlantic coast of South America.

Microsatellite markers were developed for the Florida manatee (*Trichechus manatus latirostris*) to explore finer-scale population subdivisions that might exist within populations of manatees distributed along the east and west coasts of the Florida peninsula. Low levels of allelic diversity indicated a founder effect or major population bottleneck of evolutionary significance. Cross species amplification was tested in three Sirenian species: the Antillean manatee

(*Trichechus manatus manatus*), the Amazonian manatee (*Trichechus inunguis*), and the dugong (*Dugong dugong*). This study provided nuclear DNA-based approaches to complement existing mitochondrial genetic information for these species.

Microsatellite variation in the Florida manatee population indicated that the geographically separated (but not isolated) populations from the east and the west coasts of the Florida peninsula are slightly different from each other based on microsatellite genotypic and allelic frequency distributions. High levels of gene flow and mixing occur among populations from regions present in the east and west coasts. This suggests that the Florida manatee can be considered a single Evolutionarily Significant Unit (ESU) consisting of two Management Units—the east and the west coast manatee populations. It is recommended that additional research be performed to determine the frequency of the different lineages or family units present in each geographic area, as well as the level of genetic flow between them, in order to fulfill far-reaching conservation goals.

CHAPTER 1
INTRODUCTION

The West Indian manatee (*Trichechus manatus*), which belongs to the Order Sirenia and Family Trichechidae, is a marine mammal inhabiting sub-tropical and tropical waters of the Caribbean from the southern U.S. to Brazil's northeast coast. Two species occur in the western Atlantic: the West Indian manatee (*T. manatus*) and the Amazonian manatee (*T. inunguis*) (Husar 1978). The former species occupies coastal habitats while the natural range of the latter is restricted to the Amazon basin. Two subspecies have been proposed for the West Indian form: the Florida manatee (*T. m. latirostris*), which is restricted to the Florida peninsula, and the Antillean manatee (*T. m. manatus*), which is distributed throughout the Caribbean, Central and South America (Hatt 1934; Domning & Hayek 1986).

In recent decades, manatee populations have been affected by habitat degradation (O'Shea *et al.* 1995), occasional cold weather (O'Shea *et al.* 1985), red tide outbreaks (Buergelt *et al.* 1984; O'Shea *et al.* 1991; Bossart *et al.* 1998), hunting and incidental catch (Thornback & Jenkins 1982; Mignucci-Giannoni 1990), and collisions with boats (O'Shea *et al.* 1985; Ackerman *et al.* 1995). Unfortunately, a low reproductive rate limits their ability to recover from population reductions (Thornback & Jenkins 1982; Marmontel 1995), and some populations have been reduced relative to historical levels (Lefebvre *et al.* 1989). The manatee is a specialized feeder, requiring abundant aquatic vegetation and fresh water (O'Shea & Hartley 1995), and thus habitat degradation poses an especially serious threat to their survival (Reynolds, 1995). For these reasons, the Florida manatee is listed as an endangered species (U.S. Fish and Wildlife Service 1982) and is protected under the U.S. Marine Mammal Protection Act 1972.

The physiology, social behavior and seasonal migratory patterns of the Florida manatee have been studied intensively over the past 30 years (O'Shea *et al.* 1995) and this information provides a substantial scientific foundation for management and conservation programs. For example, previous studies with protein electrophoresis indicated genetic homogeneity in manatees around the Florida peninsula. Genetic studies using allozymes by McClenaghan and O'Shea (1988) indicated that there is a strong intermixing of individuals throughout Florida, consistent with the migratory habits of this species along coastal corridors. In a more recent study using sequences of the cytochrome *b* region of the mitochondrial genome, three Florida manatee samples had the same haplotype (Bradley *et al.* 1993). Work with microsatellite markers (Tautz 1989) indicates that this may be a molecular genetic technique that will provide higher resolution of population structure. This technique has shown polymorphic and multi-allelic properties in species characterized by small population sizes and low levels of genetic variation (Taylor *et al.* 1994). Moreover, sequence homology of flanking DNA often permits the use of markers in related species.

This study was undertaken to increase our knowledge of manatee population structure and genetic diversity, and our understanding of the evolutionary relationships among geographic distinct populations. The research consists of three studies using molecular genetic techniques to elucidate the genetic diversity of the West Indian manatee (*T. manatus*).

1) The first study uses mitochondrial DNA (mtDNA) control region sequences to determine the evolutionary relatedness between geographically distinct manatee populations. This segment of the mitochondrial genome is known to accumulate mutations at a more rapid pace than coding sequences such as cytochrome *b* (Quinn 1992; Encalada *et al.* 1996), and is expected to provide a more sensitive assay for detecting diversity in the West Indian manatee. The results of this study were published in the journal *Molecular Ecology* (Garcia-Rodriguez *et al.* 1998).

2) It is recognized that mtDNA analysis has limitations and might not resolve regional population structure of manatees; therefore, a second study was undertaken that focused on the development of a finer-scale molecular technique (microsatellite DNA markers) for use in determining genetic diversity. The development and characterization of microsatellite DNA markers in the Florida manatee is documented here and the utility of these markers in three related Sirenian species is tested. The results of this work have been submitted for publication in *Molecular Ecology*.
3) The third study applies the higher resolution microsatellite markers to Florida manatee. It determines the level of genetic relatedness by examining the variation of microsatellite loci and by comparing allele frequency shifts among five locations described for the manatee population in Florida. This information is essential as effective conservation and restoration plans require clearly definable units of management.

CHAPTER 2
PHYLOGEOGRAPHY OF THE WEST INDIAN MANATEE (*Trichechus manatus*): HOW MANY POPULATIONS AND HOW MANY TAXA?

Introduction

Two species of manatee (Family Trichechidae) occur in the western Atlantic: the West Indian manatee (*Trichechus manatus*) and the Amazonian manatee (*T. inunguis*) (Husar 1978). The former species occupies coastal habitats while the natural range of the latter is restricted to the Amazon basin. However, both species require abundant aquatic vegetation and fresh water for survival. Two subspecies have been proposed for the West Indian form: the Florida manatee (*T. m. latirostris*), restricted to the Florida peninsula, and the Antillean manatee (*T. m. manatus*), distributed throughout the Caribbean, Central and South America (Hatt 1934; Domning & Hayek 1986). The Florida and the Antillean subspecies are similar in terms of external morphology, and are distinguished primarily by morphometric analyses of cranial characters (Domning & Hayek 1986).

The West Indian manatee occurs from the southeastern United States to the northeastern coast of Brazil, but this distribution is patchy. *T. manatus* typically occupies grazing pastures in shallow coastal waters and adjacent freshwater ecosystems (Best 1981), and is rare or absent from areas which lack these two habitats. Seasonal migrations are documented in Florida (Reid & O'Shea 1989; Rathbun *et al.* 1990), presumably in response to intra-annual changes in climate (Raymond 1981; Reid *et al.* 1991; Reid *et al.* 1995).

In recent decades, manatee populations have been affected by habitat degradation (O'Shea *et al.* 1995), occasional cold weather (O'Shea *et al.* 1985), red tide outbreaks (Buergelt *et al.*

1984; O'Shea *et al.* 1991; Bossart *et al.* 1998), hunting and incidental catch (Thornback & Jenkins 1982; Mignucci-Giannoni 1990), and collisions with boats (O'Shea *et al.* 1985; Ackerman *et al.* 1995). Unfortunately, a low reproductive rate limits the ability of this species to recover from population reductions (Thornback & Jenkins 1982; Marmontel 1995), and some populations have been reduced relative to historical levels (Lefebvre *et al.* 1989). The manatee is a specialized feeder and reduction of seagrass beds due to coastal development poses an especially serious threat to their survival (Reynolds, 1995). For these and other related reasons, the International Union for Conservation of Nature and Natural Resources (IUCN) recognizes *T. manatus* as a vulnerable taxon (Thornback & Jenkins 1982).

The physiology, social behavior and seasonal migratory patterns of the Florida manatee have been studied intensively over the past 30 years (O'Shea *et al.* 1995) and this information provides a substantial scientific foundation for management programs. However, the fundamental units of wildlife management, the populations, have not been adequately defined. Previous studies with protein electrophoresis indicated genetic homogeneity in manatees around the Florida peninsula; McClenaghan and O'Shea (1988) concluded that there is a strong intermixing of individuals among Florida populations, consistent with the migratory habits of this species along coastal corridors. In a more recent study using sequences of the cytochrome *b* region of the mitochondrial genome, three Florida manatee samples had the same haplotype (Bradley *et al.* 1993). To enhance population resolution, the present study focuses on the mitochondrial DNA (mtDNA) control region. This segment of the mitochondrial genome is known to accumulate mutations at a more rapid pace than coding sequences such as cytochrome *b* (Quinn 1992; Encalada *et al.* 1996), and is expected to provide a more sensitive assay of mtDNA diversity in the West Indian manatee. The resulting gene genealogies are used to illuminate aspects of evolution and biogeography that are pertinent to sirenian systematics, ecology, and wildlife management programs.

Materials and Methods

Sample Distribution

Eighty six *T. manatus* individuals were sampled from eight locations (Table 2-1) including Florida (east and west coasts, *n*=23), Chiapas and Quintana Roo, Mexico (*n*=6), the Dominican Republic (*n*=6), the southeast and northeast coasts of Puerto Rico (*n*=12), the Magdalena Basin, San Jorge River and Sinu River, Colombia (*n*=22), the Orinoco Basin and Lake Maracaibo, Venezuela (*n*=4 semi-captive individuals), Guyana (*n*=7) and the northeast coast of Brazil (*n*=6). In addition, 16 samples from *T. inunguis*, collected near Tefé (Amazonas, Brazil), were included to provide a yardstick for interpreting intraspecific partitions within *T. manatus*. Samples were collected as blood or skin biopsies from live individuals, and bone samples from carcasses (Table 2-1). In cases where captive individuals were sampled, care was taken to assure that multiple samples were not drawn from the same matriline.

DNA Isolation

Approximately 0.5 ml of blood or 1-4 g of skin were collected for genetic analysis. Skin sub-samples were minced and added to 1 ml of STE buffer (100 mM NaCl; 10 mM EDTA, pH 8.0; 50 mM Tris-HCl, pH 8.0), 12.5 µl of proteinase K (200 mg/ml) [Sigma Chemical Co.], and 0.05% [v/v] sodium dodecyl sulfate (SDS). Aliquots were incubated overnight at 50°C, then macerated with a glass homogenizer, and genomic DNA was purified with a standard phenol-chloroform procedure (Hillis *et al.* 1996). Samples from rib, vertebra and ear (periotic) bone were extracted by drilling at slow speed. Thirty to fifty mg of bone powder were incubated in extraction buffer (0.5M EDTA, 10% SDS and 25 µl of proteinase K (100 mg/ml)) at 50°C overnight (Rudin *et al.* 1995). A standard phenol/chloroform extraction was performed and the final aqueous layer was further processed as follows: a small amount of siliconized glass wool was inserted into the bottom of a 1 ml syringe barrel, which was then placed in a 15 ml conical centrifuge tube. The syringe was filled with a slurry of P6DG resin (3% w/v in water, Bio-Rad

Table 2-1. List of samples used in the study.

Species	No. of individuals	Site	Tissue sample	Observations
T. manatus	14	Florida, west coast	Blood	Wild born
T. manatus	7	Florida, east coast	Blood	Wild born
T. manatus	2	Florida , east coast	Blood	Captive born, east coast
T. manatus	4	Dominican Republic	Bone	Wild born
T. manatus	2	Dominican Republic, Baraona	Blood	Wild born
T. manatus	10	Puerto Rico, Ceiba	Blood	Wild born
T. manatus	1	Puerto Rico, Toa Baja	Blood	Wild born
T. manatus	1	Puerto Rico, San Juan	Blood	Wild born
T. manatus	1	Mexico, Quintana Roo	Blood	Wild born
T. manatus	5	Mexico, Chiapas	Blood	Wild born
T. manatus	1	Colombia, Sinu River	Blood	Wild born
T. manatus	12	Colombia, Magdalena River	Blood	Wild born
T. manatus	1	Colombia, Villa Luz Lake	Blood	Captive born
T. manatus	2	Colombia, Villa Luz Lake	Blood	Wild born
T. manatus	5	Colombia, San Jorge River	Blood	Wild born
T. manatus	1	Colombia (unknown)	Blood	Wild born
T. manatus	1	Venezuela, Maracaibo Lake	Blood	Wild born
T. manatus	1	Venezuela, Portugueza River	Skin	Wild born
T. manatus	2	Venezuela, Apure	Blood	Wild born
T. manatus	7	Guyana (unknown)	Serum	Wild born
T. manatus	6	Brazil (unknown)	Blood	Wild born
T. inunguis	3	Amazon, Amana Lake	Blood	Captive since18 April 1984
T. inunguis	1	Amazon (unknown)	Blood	Captive since 9 July 1974
T. inunguis	1	Amazon, Mamori	Blood	Captive since 24 July 1985
T. inunguis	1	Amazon, Ariau River	Blood	Captive since 16 May 1978
T. inunguis	1	Amazon (unknown)	Blood	Captive since 22 September 1977
T. inunguis	1	Amazon, Anagua Lake	Blood	Captured and release
T. inunguis	2	Amazon, Saracura Lake	Blood	Captured and release
T. inunguis	2	Amazon, Acacio Lake	Blood	Captured and release
T. inunguis	1	Amazon, Arati Lake	Skin	Captured and release
T. inunguis	2	Amazon, Acacio Lake	Skin	Captured and release
T. inunguis	1	Amazon, Apara Channel	Skin	Hunting mortality

Laboratories) and then centrifuged for three minutes at 11,000 revolutions per minute, and the liquid was removed from the bottom of the tube. Fifty µl of extract were added to the column and centrifuged for three minutes as above. Water (50 µl) was then added to the column and centrifuged again. The sample was recovered from the centrifuge tube and used as a template for PCR amplifications.

PCR Procedures

Approximately 0.1 µg of double-stranded DNA was used as a template for amplification in a 50 µl volume reaction mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.01% gelatin, 4 mM $MgCl_2$, 150 µM of each dNTP, 0.3 µM of each primer, and 2.5 units of Taq DNA polymerase (GibcoBRL). The PCR conditions used were: 94°C for 3 minutes, followed by 35 cycles at 94°C for 1 min, 47°C for 1 min, and 72°C for 1 min; a final extension period at 72°C for 1 hour completed the reaction. The heavy strand primer (CR-5: TCA CCA TCA ACA CCC AAA GC), was designed from the tRNA-Pro consensus sequences for dolphin, cow and mouse (Southern *et al.* 1988). The light strand primer (CR-4: AGA TGT CTT ATT TAA GAG GAA) was designed from a conserved segment of the control region (sequence C, figure 4, in Southern *et al.* 1988) based on 100% homology between cow and dolphin sequences. PCR-amplified products were removed from the reaction mixture with 30,000 molecular weight filters (Millipore Ultrafree-MC) following the manufacturer's protocol. DNA sequencing was accomplished in the DNA Sequencing Core at the University of Florida with the Taq DyeDeoxy terminator protocol developed by Applied Biosystems Inc. using fluorescently-labeled dideoxynucleotides. Sequences were aligned and edited with Sequencher version 3.0 (Gene Codes Corporation). Control region fragments were initially sequenced in the 5' to 3' heavy strand orientation. Ambiguous sequences, and at least one representative of each haplotype, were sequenced in the opposite direction to ensure the accuracy of nucleotide sequence designations.

Data Analysis

Nucleotide diversity (π: equation 10.5 in Nei 1987) and haplotype diversity (h: equation 8.5 in Nei 1987) within each region and overall were calculated with the computer program REAP version 4.0 (McElroy *et al.* 1992). The sequence divergence (d) within and between populations was calculated with a Kimura two-parameter algorithm (Kimura 1980) using REAP. For populations with a sample size of $n \geq 6$, a chi-square test of haplotype frequencies among regional populations was performed using the program CHIRXC (Zaykin & Pudovkin 1993), using 250 randomizations of the original data matrix to estimate an approximate probability distribution for each test (see Roff & Bentzen 1989). The proportion of genetic diversity within and among regional samples was estimated with an analysis of molecular variance (AMOVA version 1.5, Excoffier *et al.* 1992). Estimates of gene flow (migration: Nm) based on the distribution of haplotypes and the divergence among haplotypes were generated with the F_{st} analog in AMOVA (Φ_{st}). The computer program PAUP (version 3.1.1; Swofford 1993) was used to determine the number of site differences between haplotypes. Evolutionary relationships among mtDNA haplotypes were assessed with a neighbor-joining tree (Saitou & Nei 1987) using PHYLIP (version 3.5; Felsenstein 1993).

Results

A 410 basepair fragment was resolved for *T. manatus* and *T. inunguis* samples, containing 51 polymorphic sites, 47 transitions and six transversions (Table 2-2). Two positions, 328 and 332, contained both a transition and a transversion. No insertions or deletions were observed among the sequences of *T. manatus* and *T. inunguis* in this control region fragment, a finding that contrasts with control region sequence comparisons in other taxonomic groups (Lee *et al.* 1995). Sixteen haplotypes were observed within *T. manatus*, and an additional eight haplotypes were observed in the single population sample of *T. inunguis* (Table 2-3).

Table 2-2. Polymorphic sites observed in *Trichechus manatus* and *T. inunguis* haplotypes.

Haplotype	004	048	052	055	081	087	121	124	125	139	145	150	154	155	156	157	158	165	167	173	190	192	194	197	208	216	226	228	229	230	234	245	246	250	251	253	254	255	256	257	267	278	291	294	296	328	330	331	332	333	338	368
B	T	T	T	T	A	A	C	C	A	T	A	A	T	G	A	G	T	C	T	G	C	G	T	C	G	T	C	T	T	C	G	G	C	T	T	T	G	C	T	C	A	T	C	C	T	T	G	A	T	C	A	C
A	?	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
D	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	A	.	C	T	.	.
E	.	.	C	C	T	.	T	T	.	C	.	.	.	A	.	A	.	T	.	.	.	A	.	.	.	.	.	C	C	T	A	A	T	.	.	C	A	.	.	.	.	C	T	.	C	A	A	.	C	T	.	T
F	C	.	C	C	T	.	T	T	.	C	.	.	.	A	.	A	.	T	.	.	.	A	.	.	.	.	.	C	C	T	A	A	T	.	.	C	A	.	.	.	.	C	T	.	C	A	A	.	C	T	.	T
G	.	.	C	C	T	.	T	T	.	C	.	.	.	A	.	A	.	T	.	.	.	A	.	.	.	.	.	C	C	T	A	A	T	.	.	C	A	.	.	.	.	C	T	.	C	A	A	.	C	T	G	T
H	C	.	C	C	T	.	T	T	.	C	.	.	.	A	.	A	.	T	.	.	.	A	.	.	.	.	.	C	C	T	A	A	T	.	.	C	A	.	.	.	.	C	T	.	C	A	A	.	C	T	G	T
I	?	.	C	C	T	.	T	T	.	C	.	.	.	A	.	A	.	T	.	.	.	A	.	T	.	.	.	C	C	T	A	A	T	.	.	C	A	.	.	.	.	C	T	.	C	C	A	.	C	T	.	T
J	.	.	C	C	T	.	T	T	.	C	.	.	.	A	.	A	.	T	.	.	.	A	.	.	.	.	.	C	C	T	A	A	T	.	.	C	A	.	.	.	.	C	T	.	C	C	A	.	C	T	.	T
K	.	.	C	C	T	.	T	T	.	C	.	.	.	A	.	A	.	T	.	.	.	A	.	.	.	.	.	C	C	T	A	A	T	.	.	C	.	.	.	.	.	C	T	.	C	C	A	T	C	T	.	T
L	?	.	.	.	T	.	.	T	.	.	.	.	.	A	G	A	C	.	.	.	.	A	C	.	A	C	T	C	.	.	A	.	.	.	.	.	.	T	.	.	.	C	T	T	C	.	.	.	?	T	.	T
M	.	.	.	.	T	.	.	T	.	.	.	.	.	A	G	A	C	.	.	.	.	A	C	.	.	C	T	C	.	.	A	.	.	.	.	.	.	T	.	.	.	C	T	T	C	.	.	.	C	T	.	T
N	.	.	.	.	T	.	.	T	.	.	.	.	.	A	G	A	C	.	.	.	.	A	C	.	.	C	T	C	.	.	A	.	.	C	.	.	.	T	.	.	.	C	T	T	C	.	.	.	G	T	.	T
O	.	.	.	.	T	.	.	T	T	.	.	.	.	A	G	A	C	.	.	.	.	A	C	.	.	C	T	C	.	.	A	.	.	C	.	.	.	T	.	.	.	C	T	T	C	.	.	.	G	T	.	T
P	.	C	C	C	.	G	.	T	.	.	G	.	C	A	.	.	.	.	C	A	T	A	.	.	A	.	T	C	.	.	.	.	.	.	C	C	A	.	C	T	C	C	T	.	.	.	.	.	.	.	.	T
Q	.	C	C	C	.	G	.	T	.	.	G	.	C	A	.	.	.	.	C	A	T	A	.	.	.	.	T	C	.	.	.	.	.	.	C	.	A	.	C	T	C	C	T	.	.	.	.	.	.	.	.	T
R	.	.	C	C	.	G	.	T	.	.	G	.	C	A	.	.	.	.	C	A	T	A	.	.	.	.	T	C	.	.	.	.	.	.	C	.	A	.	C	T	C	C	T	.	.	.	.	.	.	.	.	T
S	.	.	C	C	.	G	.	T	.	.	G	.	C	A	.	.	.	.	C	A	T	A	.	.	.	.	T	C	.	.	.	.	.	.	C	C	A	.	C	T	C	C	T	.	.	.	.	.	.	.	.	T
T	.	C	C	C	.	G	.	T	.	.	G	.	C	A	.	.	.	.	C	A	T	A	.	.	.	.	T	C	.	.	.	.	.	.	C	C	A	.	C	T	C	C	T	.	.	.	.	.	.	.	.	T
U	.	C	C	C	.	G	.	T	.	.	G	G	C	A	.	.	.	.	C	A	T	A	.	.	.	.	T	C	.	.	.	.	.	.	C	.	A	.	C	T	C	C	T	.	.	.	.	.	.	.	.	T
V	.	C	C	C	.	G	.	T	.	.	G	.	C	A	.	.	.	.	C	A	T	A	.	.	.	.	T	C	.	.	.	.	.	.	C	.	A	.	C	.	C	C	T	T	.	.	.	.	.	.	.	T
W	.	C	C	C	.	G	.	T	.	.	G	.	C	A	.	.	.	.	C	A	T	A	.	.	.	.	T	C	.	.	.	.	.	.	C	.	A	.	C	.	C	C	T	.	.	.	.	.	.	.	.	T
X	.	C	C	C	.	G	.	T	.	.	G	.	C	A	.	.	.	.	C	.	T	A	.	.	.	.	T	C	.	.	.	.	.	.	C	.	A	.	C	.	C	C	T	T	.	.	.	.	.	.	.	T

These polymorphisms include 47 transitions and six transversions. Both transitions and transversions are found in sites 328 and 332. Haplotypes A-O are *T. manatus*, haplotypes Q-X are *T.inunguis*, and P represents possible hybrids (see Discussion). Question marks indicate unresolved nucleotides. Representative sequences (from haplotypes A, J, M, and T) have been submitted to GeneBank, Accession nos: AF046157, AF46159 and A046160, respectively.

Table 2-3. Distribution of manatee mtDNA haplotypes.

Species	Cluster	Haplotype	Florida (*n*=23)	Puerto Rico (*n*=12)	Dominican Republic (*n*=6)	Mexico (*n*=6)	Colombia (*n*=22)	Venezuela (*n*=4)	Guyana (*n*=7)	Brazil (*n*=22)
T. manatus	1	A	23	5	2					
		B		7	4					
		C					4			
		D					1			
	2	E					7			
		F					2			
		G					5			
		H					2			
		I						1		
		J				6	1	2		
		K						1		
	3	L							1	
		M							1	6
		N							1	
		O							1	
		P							3	
T. inunguis	4	Q								1
		R								1
		S								3
		T								5
		U								1
		V								2
		W								1
		X								2

Cluster 1 represents haplotypes from Florida, the Caribbean and Colombia. Cluster 2 represents haplotypes from Mexico, Colombia and Venezuela. Cluster 3 represents haplotypes from Guyana and Brazil. Cluster 4 represents haplotypes from Brazil and Guyana. The letters A to O represent haplotypes from *T. manatus*. The letters Q to X represent haplotypes from *T. inunguis*. The letter P represents possible hybrids between *T. manatus* and *T. inunguis*. Brazilian samples (*n* = 22) include the coastal *T. manatus* (*n* = 6) and the Amazonian form (*n* = 16).

The level of mtDNA diversity varied widely between regional samples (Table 2-4). *T. manatus* samples from Florida (*n*=23), Mexico (*n*=6) and Brazil (*n*=6) contained no polymorphism, while samples from Colombia and adjacent Guyana contained more than 20 polymorphic sites. Overall haplotype diversity within *T. manatus* was relatively high (*h*=0.839). Overall nucleotide diversity was relatively high for a large vertebrate (π=0.040), a finding which may be attributed to the deep evolutionary partition observed among clusters of haplotypes (see below). Maximum sequence divergence among pairwise comparison of haplotypes (*d*=0.074) was high relative to the level observed in congeneric control region comparisons in other marine mammals (Rosel *et al.* 1994; Baker *et al.* 1994).

Table 2-4. Haplotype (*h*) and nucleotide diversity (π) for manatees *Trichechus manatus* and *T. inunguis.*

Population	Sample size (*n*)	Haplotype diversity (*h*)	Nucleotide diversity (π)
T. manatus			
Overall	86	0.839 ± 0.028	0.040
Florida	23	0.000	0.000
Puerto Rico	12	0.530 ± 0.076	0.001
Dominican Republic	6	0.533 ± 0.172	0.001
Mexico	6	0.000	0.000
Colombia	22	0.831 ± 0.047	0.027
Guyana	7	0.857 ± 0.137	0.044
Brazil	6	0.000	0.000
T. inunguis			
Brazil	16	0.875 ± 0.059	0.005

The Venezuelan population ($n = 4$) is not included in location-specific estimates of genetic diversity due to small sample size, but is included in overall estimates of genetic diversity. The Guyana sample includes three putative hybrids (see Discussion).

Among the 16 haplotypes in *T. manatus* samples, twelve were observed in only one sample location (private or endemic haplotypes; Slatkin 1985a) and four were shared among sample sites (Table 2-3). Despite the sharing of haplotypes among locations, there was strong geographic structuring of mtDNA diversity (Figure 2-1). Haplotype frequencies were significantly different in 20 of 21 pairwise comparisons (Table 2-5), and analysis of molecular variance (AMOVA) indicates that 79.8% of the total mtDNA variation in *T. manatus* was partitioned among sample locations.

Migrations (in the sense of gene flow) between locations can be expressed as *Nm*, the number of migrants per generation (Slatkin 1985b). In general, values of *Nm* >1-4 indicate that gene flow is sufficient to homogenize haplotype frequencies among locations. Values below this threshold may indicate that populations will diverge through the process of genetic drift (but see Allendorf 1983). Estimates of manatee migration among localities based on the F_{st} analog in AMOVA (Φ_{st}), using equation $Nm = 1/2(1/F_{st}-1)$ from Takahata & Palumbi (1985), ranged from $Nm = 0.0\text{-}3.5$ (Table 2-5).

Phylogenetic and phenetic analyses reveal three primary mtDNA lineages within *T. manatus*, separated from each other by sequence divergence estimates of d=0.04-0.07 (Table 2-6). Haplotypes A-D, corresponding to all samples from Florida, Puerto Rico, the Dominican Republic, and five of 22 specimens from Colombia, constitute the "Florida and West Indies Cluster" or Cluster 1 in Figure 2-2. A second grouping, defined as "the Gulf of Mexico to Caribbean rivers of South America Cluster" or Cluster 2 (haplotypes E-K), include all samples from Mexico, 17 of 22 specimens from Colombia, and all four samples from Venezuela. The third grouping, defined as the "northeast Atlantic coast of South America Cluster" or Cluster 3 (haplotypes L-O), includes all samples from Brazil and all but three individuals from Guyana (see below). Bootstrap support for these distinct lineages ranges from 87-100% in the neighbor-

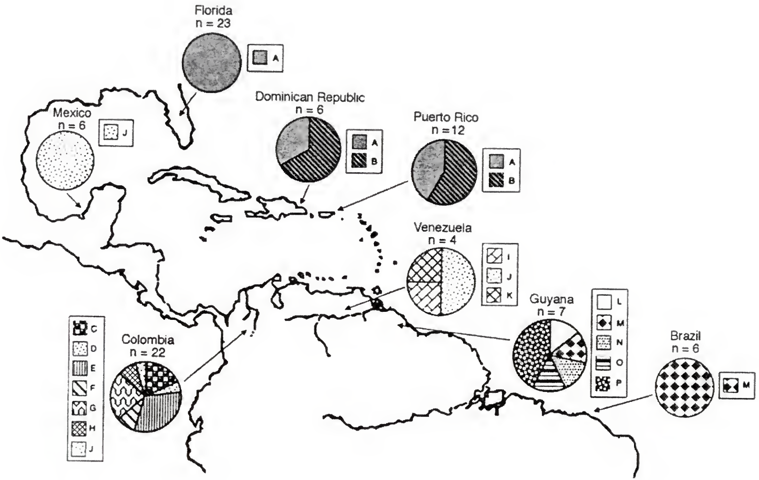


Figure 2-1. Distribution of manatee samples. Pie charts indicate the frequency of haplotypes in each location.

Table 2-5. Population parameters for *Trichechus manatus*.

Population	Florida	Puerto Rico	Dominican Republic	Mexico	Colombia	Guyana	Brazil
Florida	–	0.268	0.114	0.000	0.145	0.115	0.000
Puerto Rico	17.38 $P<0.001$	–	3.468	0.007	0.218	0.220	0.009
Dominican Republic	18.46 $P=0.002$	**0.117** **$P=1.000$** **NS**	–	0.005	0.274	0.352	0.006
Mexico	30.00 $P<0.001$	18.00 $P<0.001$	12.00 $P=0.002$	–	3.539	0.300	0.000
Colombia	46.00 $P<0.001$	34.00 $P<0.001$	28.00 $P<0.001$	23.00 $P<0.001$	–	0.566	0.296
Guyana	31.00 $P<0.001$	19.00 $P<0.001$	13.00 $P=0.011$	13.00 $P=0.001$	29.00 $P<0.001$	–	1.132
Brazil	30.00 $P<0.001$	18.00 $P<0.001$	12.00 $P=0.005$	12.00 $P=0.004$	28.00 $P<0.001$	9.55 $P=0.015$	–

Above diagonal: migration rates (*Nm*) between sample locations based on Φ_{st} estimator. Below diagonal: pairwise haplotype frequency comparisons based on χ^2 statistics. Chi-Square and *P*-values appear in bold for one population pair, which did not differentiate (NS, non-significant). The Venezuelan population is excluded from the analysis because of small sample size ($n = 4$).

Table 2-6. Genetic distances between haplotypes calculated with the Kimura-two parameter algorithm.

Haplotype	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X
A	0.0000																							
B	0.0026	0.0000																						
C	0.0052	0.0073	0.0000																					
D	0.0130	0.0148	0.0073	0.0000																				
E	0.0726	0.0659	0.0685	0.0607	0.0000																			
F	0.0726	0.0685	0.0712	0.0633	0.0024	0.0000																		
G	0.0754	0.0685	0.0712	0.0633	0.0024	0.0049	0.0000																	
H	0.0754	0.0712	0.0738	0.0659	0.0049	0.0024	0.0024	0.0000																
I	0.0752	0.0717	0.0744	0.0662	0.0051	0.0051	0.0077	0.0077	0.0000															
J	0.0724	0.0658	0.0684	0.0605	0.0024	0.0049	0.0049	0.0074	0.0026	0.0000														
K	0.0726	0.0659	0.0685	0.0607	0.0074	0.0098	0.0098	0.0123	0.0077	0.0049	0.0000													
L	0.0586	0.0539	0.0565	0.0539	0.0539	0.0539	0.0565	0.0565	0.0579	0.0538	0.0539	0.0000												
M	0.0559	0.0502	0.0528	0.0502	0.0502	0.0528	0.0528	0.0554	0.0552	0.0501	0.0502	0.0025	0.0000											
N	0.0587	0.0529	0.0555	0.0555	0.0555	0.0580	0.0580	0.0607	0.0607	0.0554	0.0555	0.0075	0.0049	0.0000										
O	0.0616	0.0555	0.0581	0.0581	0.0581	0.0608	0.0608	0.0634	0.0635	0.0580	0.0581	0.0100	0.0074	0.0024	0.0000									
P	0.0614	0.0605	0.0528	0.0605	0.0713	0.0739	0.0739	0.0766	0.0773	0.0712	0.0766	0.0699	0.0712	0.0739	0.0767	0.0000								
Q	0.0559	0.0554	0.0476	0.0554	0.0713	0.0739	0.0739	0.0766	0.0773	0.0712	0.0766	0.0699	0.0659	0.0686	0.0714	0.0049	0.0000							
R	0.0531	0.0528	0.0451	0.0528	0.0686	0.0713	0.0713	0.0739	0.0746	0.0685	0.0739	0.0673	0.0633	0.0660	0.0687	0.0073	0.0024	0.0000						
S	0.0559	0.0554	0.0476	0.0554	0.0660	0.0686	0.0686	0.0713	0.0718	0.0659	0.0713	0.0699	0.0659	0.0686	0.0714	0.0049	0.0049	0.0024	0.0000					
T	0.0586	0.0580	0.0502	0.0580	0.0686	0.0713	0.0713	0.0739	0.0746	0.0685	0.0739	0.0726	0.0685	0.0713	0.0740	0.0024	0.0024	0.0049	0.0024	0.0000				
U	0.0586	0.0580	0.0502	0.0580	0.0739	0.0766	0.0766	0.0792	0.0801	0.0738	0.0792	0.0726	0.0685	0.0713	0.0740	0.0073	0.0024	0.0049	0.0073	0.0049	0.0000			
V	0.0559	0.0554	0.0476	0.0554	0.0713	0.0739	0.0739	0.0766	0.0773	0.0712	0.0766	0.0646	0.0607	0.0634	0.0661	0.0098	0.0049	0.0073	0.0098	0.0073	0.0073	0.0000		
W	0.0531	0.0528	0.0451	0.0528	0.0686	0.0713	0.0713	0.0739	0.0746	0.0685	0.0739	0.0673	0.0633	0.0660	0.0687	0.0073	0.0024	0.0049	0.0073	0.0049	0.0049	0.0024	0.0000	
X	0.0531	0.0528	0.0451	0.0528	0.0686	0.0713	0.0713	0.0739	0.0746	0.0685	0.0739	0.0619	0.0580	0.0608	0.0635	0.0123	0.0073	0.0098	0.0123	0.0098	0.0098	0.0024	0.0049	0.0000

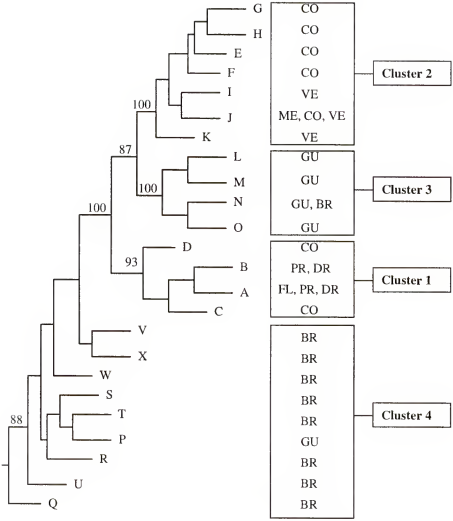


Figure 2-2. Neighbor-joining tree describing the relationships among the three clusters of mtDNA haplotypes in *T. manatus*. Cluster 1: The Florida and West Indies (haplotypes A-D), Cluster 2: the Caribbean coast and rivers of Central America to the northern Atlantic coast of South America (haplotypes E-K) and Cluster 3: the northeast Atlantic coast of South America (haplotypes L-O). Bootstrap values for critical nodes are derived from parsimony analysis and based on 100 replicates.

joining analysis. Despite the overlap of these lineages in Colombia, the results indicate strong geographic structuring among mtDNA lineages in *T. manatus*, defining three phylogeographic units which are not concordant with the accepted subspecies designation.

T. manatus and *T. inunguis* samples are distinguished by *d*=0.04-0.07 (Table 2-6), values which parallel the range of divergences among the three geographic clusters of *T. manatus* (*d*=0.04-0.07). Hence the anticipated genetic distinction of *T. inunguis* and *T. manatus* was not supported by mtDNA data. Furthermore, three individuals from Guyana, identified as *T. manatus* by field researchers, contained haplotype P which is affiliated with *T. inunguis* in phylogenetic analyses. This finding was unexpected because *T. inunguis* is not known to occur outside the Amazon Basin.

Discussion

Manatees are characterized by significant haplotype frequency shifts between most locations, low mtDNA diversity in some locations, and strong (but incomplete) geographic partitioning of three relatively deep mtDNA lineages corresponding approximately to the West Indies (and Florida), the Caribbean mainland coast, and the Atlantic coast of South America. What aspects of climate, geography, and natural history could produce these phylogeographic patterns? Whitehead (1977) suggested that the range of *T. manatus* falls within the northern and southern limits of the 24°C mean annual isotherm. The manatee's low metabolic rate (15-22% of predicted weight-specific values) and high thermal conductance, have prompted suggestions that the manatees could not survive winter water temperatures in latitudes north of central Florida (Irvine 1983). Shallow coastal areas, shelter from oceanic wave action, and availability of vegetation and freshwater are also important elements of manatee habitat (Lefebvre *et al.* 1989).

Population Genetic Structure in *T. manatus*

Patches of manatee habitat are characterized by population-level separations, yet dispersal events over great geographic scales have occurred, as indicated by sharing of haplotypes A and J among widely separated locations (Table 2-3). How can these apparently conflicting observations be explained? One consideration is the preference of *T. manatus* for near-shore habitat. These animals are rarely observed in deep water, where appropriate food and freshwater are unavailable and where the risk of shark predation may be high (Mou Sue *et al.* 1990). Therefore the strict coastal habitat of *T. manatus* may be a primary regulator of population partitions. A second consideration is the evidence that long-distance movements are rare, based on direct observations. Suspected strays from Florida have been recorded as far north as Rhode Island (Reid 1996), as far east as the Bahamas (Odell *et al.* 1978; Domning & Hayek 1986) and as far west as Texas (Wright 1997). Reynolds and Ferguson (1984) sighted two manatees 61 kms northeast of the Dry Tortugas, and suggested that they could be wanderers from Florida, Cuba, or the Yucatan Peninsula. A third consideration is the temporal scale and frequency of long-distance colonization. Population genetic theory and empirical studies both demonstrate that even rare migration events can be sufficient to homogenize populations (Slatkin 1985b). Hence the population structure of *T. manatus* probably reflects the balance between habitat requirements which limit dispersal, and the swimming ability of manatees which permits rare colonization events.

Does this confluence of genetic and distribution data allow predictions about population structure in unsurveyed manatee aggregates? While the exact relationships among populations may be difficult to predict, the mtDNA data support a model in which manatee aggregates separated by large tracts (100s to 1000s of km) of inappropriate habitat are probably distinct demographic units. This conclusion must be considered provisional, however, because mtDNA surveys include only the maternal lineages. Evidence of sex-specific dispersal has been reported

(Bengtson 1982) and nuclear DNA assays will be necessary to evaluate this aspect of manatee population structure.

Low mtDNA Diversity as an Indicator of Population History

Samples from east and west Florida (*n*=23) contained only a single haplotype, so it was not possible to determine whether population structure exists across the Florida peninsula. However, the relatively continuous distribution of manatee habitat in southern Florida, and the swimming capacity of these animals (Reid 1996; Wright 1997), diminishes the possibility of strong reproductive isolation on this geographic scale (McClenaghan & O'Shea 1988). What could explain the low level of mtDNA diversity in Florida manatees? Two historical explanations merit consideration. First, perhaps the Florida population passed through a bottleneck (severe reduction in population size) in recent evolutionary time. Any number of biological or physical stresses could induce this phenomenon, including extreme winter seasons (O'Shea *et al.* 1985; Ackerman 1995) and epizootics (Buergelt *et al.* 1984; O'Shea *et al.* 1991, Bossart *et al.* 1998). For example, in 1996 about 5-10% of the Florida manatees perished due to brevetoxin exposure caused by a severe red tide (Bossart *et al.* 1998). Alternatively, the lack of mtDNA diversity may be the result of a recent colonization event from the West Indies. Geochemical evidence indicates that the tropical Atlantic was 5°C cooler during the most recent (Wisconsin) glacial period, approximately 18,000 to 12,000 years before Pleistocene (BP) (Guilderson *et al.* 1994), and paleontological records show that the temperate fauna of the mid-Atlantic bight (Massachusetts to North Carolina, USA) extended south of the Florida peninsula during this interval (Hedgpeth 1954). Hence, it is unlikely that a manatee population persisted in Florida through the Wisconsin glacial period. Florida was probably colonized (or recolonized) during the last 12 thousand years, an interval too short to allow accumulation of equilibrium levels of diversity in the mitochondrial genome. The phylogenetic affiliation of Florida samples with the West Indies cluster, and the fixation of one haplotype which also occurs in the Dominican Republic and Puerto Rican populations, indicate this geographically proximate source of colonizers.

No mtDNA diversity was observed for the Brazilian samples of *T. manatus* (*n*=6), contrasting with the other populations surveyed in South America (Guyana with five haplotypes, Venezuela with three, and Colombia with seven). Like the Florida example discussed above, the Brazilian population occupies a high-latitude extreme of the range, and is known to have a relatively small population size (Domning 1982a; Lefebvre *et al.* 1989). These factors could certainly influence the level of observed genetic diversity. However, the alternate explanation of recovery from a recent bottleneck effect also bears consideration. Manatee populations in Brazil (both *T. manatus* and *T. inunguis*) have been commercially exploited for 300 years (Domning 1982a). While historical records are scarce, current populations are almost certainly a fraction of pre-exploitation levels. Hence three centuries of exploitation might also contribute to the low haplotype diversity observed in Brazilian samples of *T. manatus*.

Phylogeography of *T. manatus*

The mtDNA phylogeny for *T. manatus* includes three primary lineages corresponding to Florida and the West Indies (Cluster 1), the Gulf of Mexico and Caribbean rivers of South America (Cluster 2), and the northeast Atlantic coast of South America (Cluster 3; see Figure 2-2). These groupings are not completely segregated, as haplotypes from Clusters 1 and 2 co-occur in Colombia (Table 2-3).

The sequence divergence between the three primary *T. manatus* lineages is comparable to the level of control region divergence between genera of whales (Baker *et al.* 1993), raising the possibility that these partitions reflect deep evolutionary separations (see Moritz 1994a). How old are these three lineages? While a control region clock for manatees is unavailable, estimated rates of change reported for terrestrial mammals range from approximately 8-15% per million years (Myr) between lineages (Vigilant *et al.* 1991; Stewart & Baker 1994). Estimated rates for baleen whales, based on fossil calibrations, are approximately 1% per Myr (Hoelzel *et al.* 1991; Baker *et al.* 1993) and the estimated rate for dugong (*Dugong dugong*, a Pacific sirenian) is 2% per Myr based on fossil evidence (Tikel 1997). Using the provisional range of values for terrestrial mammals, the three lineages in *T. manatus* diverged on the order of 0.5-1 Myr BP. The time frame based on the marine mammals (1-2% per Myr) is approximately 0.5-3.5 Myr BP. This slower molecular clock (and correspondingly greater time estimate) may be more appropriate for manatees, as 1) long generation time and low metabolic rate are correlated with slower molecular clocks in other vertebrates (Avise *et al.* 1992; Martin & Palumbi 1993) and 2) this range of estimates is calibrated for another member of the Sirenia. Notably, the range of estimates based on this clock bracket the period inferred from the fossil record for the origin of *T. manatus* (within the last 4 Myr; Domning 1982b).

What could account for the distribution of the three primary branches in the mtDNA phylogeny? The rapid lineage sorting of maternally-inherited mtDNA diminishes the possibility that all three lineages co-existed in the same populations through the last few million years. A more likely scenario involves isolation for an extended period followed by recent range extensions and admixture. Because manatees do not readily cross wide stretches of open water, the isolation of a Florida-West Indies cohort (Cluster 1) can be explained in terms of habitat distribution and the isolation of the Greater Antilles from continental coastlines. The separation of Caribbean-Continental (Cluster 2) and South American (Cluster 3) cohorts (with a putative break in the vicinity of Venezuela and Guyana) is consistent with the boundaries of marine biogeographic provinces. In particular, Briggs (1974) considers the coastal habitats of Venezuela to be the southernmost outpost of the Caribbean province. Hence the phylogeographic break between Caribbean-Continental and South American mtDNA lineages is concordant with a discontinuity in the distribution of other marine faunas.

The spread of the West Indies mtDNA lineage into Florida is probably a range expansion after the Wisconsin glaciation. The extension of the West Indies lineage into South America is likely to have occurred in recent evolutionary time, based on the similarity of mtDNA haplotypes A and B in the West Indies versus C and D in Colombia. While a specific route of colonization cannot be inferred from the molecular data, it is notable that archaeological and early historic evidence indicate that manatees occurred in the Lesser Antilles during the age of European exploration (Lefebvre *et al.* 1989). Thus, the link between haplotypes observed in the West Indies (Puerto Rico and the Dominican Republic) and the northern rim of South America (Colombia) may have been established by now-extinct populations that inhabited the Lesser Antilles.

Manatee Evolution and Systematics

Fossil evidence indicates that the ancestor of extant manatees arose during the Miocene, possibly in coastal rivers and lagoons of South America, and never expanded beyond the Atlantic Ocean and adjacent tributaries (Domning 1982b). The Amazonian *T. inunguis* may have arisen in isolation after the Miocene origin of the Andes (Domning 1982b). This species is distinguished from *T. manatus* by a range of morphological characters including lack of nails on the pectoral flippers, reduced number of dorsal vertebrae, smaller and more complex molars, and thickened supraoccipital (Domning & Hayek 1986). In addition, *T. inunguis* has a higher diploid chromosome number (56 compared to 48 in *T. manatus*; Loughman *et al.* 1970; White *et al.* 1976). *T. inunguis* is distributed along the Amazon River and adjacent coastal estuaries, and it is not known what factors maintain the apparent parapatry of *T. inunguis* and *T. manatus* at the mouth of the Amazon River (Domning 1981).

The mtDNA phylogeny of Western Atlantic manatee species does not readily align with the current taxonomy of *Trichechus* spp. The control region phylogeny contains four primary branches, including three in *T. manatus* and one in *T. inunguis*. While this does not necessarily contradict the species status of the Amazonian manatee, it does indicate that the two putative western Atlantic species shared a maternal ancestor more recently than was previously suspected.

Molecular clocks must be interpreted with caution, but the range of dates estimated from mtDNA data (2-4 Myr based on the dugong calibration of 2%/Myr) are consistent with a Pliocene (rather than a Miocene) origin for the Amazonian form.

Subspecies designations based on cranial characters in *T. manatus* also do not coincide with the intraspecific partitions based on mtDNA sequences. Domning and Hayek (1986) used the name *T. m. latirostris* to distinguish the Florida morphotype from *T. m. manatus* populations through the rest of the range. In contrast, the mtDNA data link the Florida manatee with those from the West Indies. The molecular data do not strictly contradict the accepted taxonomic assignments, but bolster the hypothesis that the Florida form is a very recent derivative of West Indies populations. As in the comparison of *T. manatus* and *T. inunguis*, the mtDNA data are useful for placing an approximate evolutionary timeframe on the documented morphological differentiation.

One surprising result is the phylogenetic affiliation of a haplotype of *T. manatus* from the Guyana population with the endemic Amazonian form, *T. inunguis*. These findings invoke a range of interesting possibilities, including an enormous range expansion of *T. inunguis*, retention of an ancestral haplotype since the *manatus-inunguis* split, a re-alignment of manatee taxonomy, or a hybrid zone in northern South America. Nuclear DNA sequence and chromosome analyses will doubtless prove useful in sorting out these possibilities, but a simpler explanation must be considered here. All three individuals with the putative *T. inunguis* haplotype came from a system of freshwater canals located within the Guyana Zoological Gardens. While zoo records are incomplete, manatees were stocked into this area thirty years ago from adjacent regions to enhance the zoo collection and to control aquatic vegetation (Allsopp 1969). It is possible that a few *T. inunguis* were acquired and released with *T. manatus*. However, the contemporary specimens used for genetic analysis were identified during the preparation of this report as *T. manatus* based on morphometric characters (P. MacWilliams, pers. comm.). This finding raises the possibility of human-induced hybridization between *T. manatus* and *T. inunguis* in Guyana.

Notably, *T. inunguis* has also been introduced into manatee habitats in Panama (Mou Sue *et al.* 1990), invoking the possibility of a second hybrid zone.

Overall, the mtDNA data prompt a recognition that taxonomic designations for *Trichechus* may not reflect evolutionary partitions. Although the information from a single line of evidence (such as mtDNA) is not sufficient to overturn robust taxonomic classifications based on a suite of morphological characters, it is certainly sufficient to identify points for further inquiry. Research efforts in progress (L. Parr, S. Fain, and D. Duffield, pers. comm.) should clarify the higher level systematics of manatees. Nuclear DNA assessment and morphometric analyses, as well as additional sampling, are also desirable to resolve remaining evolutionary and systematic issues.

Conservation Implications

Intensive research in Brazil, Mexico, the United States, Puerto Rico and elsewhere has provided a scientific foundation for efforts to preserve remaining manatee populations (O'Shea *et al.* 1995). The data presented here enhance this conservation foundation in at least three ways. First, mtDNA haplotype frequency comparisons provide the first robust assessment of the interaction between habitat restrictions (which presumably limit movement) and dispersal events in defining population genetic units. Long-distance migration does occur, but is evidently not sufficient to homogenize populations separated by large tracts of inappropriate habitat. The results from surveyed manatee populations directly confirm the demographic independence of isolated populations, and (perhaps more importantly) provide an approximate yardstick to define management units throughout the range of *T. manatus*.

A second conservation consideration is that previously unrecognized evolutionary partitions may exist within *T. manatus*. The mtDNA data alone are not sufficient to resolve this question, but provide strong motivation for additional investigations. If the separations defined in a mtDNA phylogeny are supported with other lines of evidence, then conservation priorities may be realigned to include three or more taxonomic and evolutionary units within *T. manatus*.

Finally, a relatively thorough catalog of genetic diversity in manatees can provide a foundation for forensic investigation. Based on the highly structured nature of manatee populations, it is feasible to identify suspected manatee products (such as meat, organs, bones etc.) to species and regions of origin. This would enhance the arsenal for enforcement of wildlife protection regulations, illustrating the value of range-wide genetic inventories of endangered species.

CHAPTER 3
ISOLATION AND CHARACTERIZATION OF MICROSATELLITE DNA MARKERS IN THE FLORIDA MANATEE (*Trichechus manatus latirostris*) AND THEIR APPLICATION IN SELECTED SIRENIAN SPECIES

The West Indian manatee (*Trichechus manatus*) is a marine mammal inhabiting sub-tropical and tropical waters of the Caribbean from the southern U.S. to Brazil's northeast coast. The species is endangered due to habitat loss, human-related mortality and low reproductive rate (Lefebvre *et al.* 1989). Two subspecies are recognized, the Florida manatee (*T. m. latirostris*) and the Antillean manatee (*T. m. manatus*) (Domning & Hayek 1986). Abundant biological and ecological data for both sub-species have been collected and the information has become the basis for management and conservation programs. However, for planning and implementing biologically sound management programs for this marine mammal, knowledge of the amount of genetic diversity present and a thorough understanding of the evolutionary relationships among geographic populations are essential.

Genetic studies employing allozymes (McClenaghan & O'Shea 1988) and mitochondrial DNA (mtDNA) (Bradley *et al.* 1993; Garcia-Rodriguez *et al.* 1998) have identified low levels of genetic diversity, and failed to resolve population structure for the Florida manatee. Therefore, a molecular genetic technique with the potential for higher resolution of population structure is needed. Microsatellite loci (Tautz 1989) have been proven to exhibit polymorphic and multi-allelic properties in species characterized by small population sizes and low level of genetic variation (Taylor *et al.* 1994). Moreover, sequence homology of flanking DNA often permits the use of markers in related species. In this study the development and characterization of microsatellite DNA markers in the Florida manatee is documented and the utility of these markers in three closely related Sirenian species is tested.

Two methodologies were used to generate microsatellite-enriched libraries for *T. m. latirostris*. Four enriched libraries were produced by Genetic Identification Services (Chatworth, California) using proprietary magnetic bead capture technology. An additional library was constructed and screened for polymorphic loci following a modified protocol of Armour *et al.* 1994. Approximately 50 μg of manatee genomic DNA were digested with *Sau* 3A I (Life Technologies, Rockwell, MD), gel-fractionated to isolate 0.4-1.0 kbp fragments and ligated to *Sau* 3A I linkers. PCR amplifications using *Sau*-L-A primer were performed in a 100 μl PCR reaction containing: 15 ng of purified DNA, 10 μl of 10X PCR buffer (Promega, Madison, WI), 10 μl of 25 mM $MgCl_2$, 10 μl of 2 mM dNTPs, 10 μl of 10 μM *Sau*-L-A primer, and 5 μl of 0.5 U/μl *Taq* polymerase. The following amplification program was used: 94°C for 3 min, 30 cycles of 94°C for 45 sec, 68°C for 45 sec, and 72°C for 1.5 min, followed by 72°C for 10 min. Purified PCR products were denatured by alkali treatment and hybridized to nylon filters containing $(CA)_n$ oligonucleotide repeats. Hybridization was performed overnight at 65°C and 5 μl of the recovered hybridized molecules were used for a 100μl PCR amplification of microsatellite-enriched genomic DNA fragments following the amplification and PCR conditions described above. PCR products were directly ligated to pCR®2.1 (Invitrogen, Carlsbad, CA) followed by transformation into INVαF' One Shot™ competent cells (Invitrogen). A total of 186 colonies was screened for $(CA)_n$-containing inserts with alkaline phosphatase conjugated $(TG)_n$ oligomer and a chemiluminescent detection system (FMC® BioProducts Corp., Rockland, ME). Following a secondary screening, 60 positive colonies were sequenced.

Between the two sets of libraries, primers were designed in the flanking regions of 61 microsatellite bearing clones using OLIGO 5.1 (National Biosciences, Molecular Biology Insights, Inc., Cascade, CO). Amplification was performed in a Biometra® UNO II thermal cycler using the following conditions: 94°C for 2 min; 34 times the following cycle: 94°C for 30 sec, 54°C for 30 sec, 72°C for 30 sec; and a final extension at 72°C for 10 min.

Fifteen sets of primers amplified consistently scorable fragments of expected size with Florida manatee genomic DNA (Table 3-1). These markers were screened in 50 manatees collected throughout the Florida peninsula. Eight of the 15 loci were polymorphic in this initial survey and overall levels of heterozygosity averaged 41.2%. Low levels of allelic diversity were observed in the Florida manatee. The maximum number of alleles identified at the most polymorphic locus (TmaE11) was six and the average number of alleles observed at polymorphic loci was 2.9. This paucity of genetic diversity is indicative of a founder effect or major population bottleneck of evolutionary significance (see Garcia-Rodriguez *et al.* 1998). In comparison to published reports on related terrestrial (African elephant; Nyakaana and Arctander 1999) and marine mammals (Atlantic walrus, Andersen *et al.* 1998; right whale, Waldick *et al.* 1999), the Florida manatee exhibited considerably less allelic diversity. Indeed, this study reports one of the lowest levels of genetic diversity observed in species-specific microsatellite markers.

Regardless of the low level of genetic diversity, the markers appear to provide an enhanced perspective of the population structure. Preliminary analysis of nearly 223 samples from Florida suggests some level of allelic differentiation between the east and west coast populations, which has not been previously detected with allozymes or mtDNA (McClenaghan & O'Shea 1988; Garcia-Rodriguez *et al.* 1998).

Cross species amplification was tested in three Sirenian species: the Antillean manatee (*T. m. manatus*), the Amazonian manatee (*T. inunguis*), and the dugong (*Dugong dugong*). Eleven of 15 markers were polymorphic for the Antillean and the Amazonian manatee (Table 3-2). At least nine markers were polymorphic in the dugong; however, estimates of polymorphism are likely to be underestimated due to the small sample size. This suite of markers appears to be ideal for identification of population structure and possibly pedigree analysis; in all four Sirenian species, thus; providing nuclear DNA-based approaches to complement existing mtDNA genetic information for these vulnerable species.

Table 3-1. Characteristics of 15 *Trichechus manatus latirostris* microsatellite markers.

Locus	Size range (bp)	Repeat type (and length)	No. of alleles	H_o	H_e	Primer sequences (5' to 3')	Accession number
TmaA01	107	$(TA)_3(CA)_3CG(CA)_7$	1	0.000	0.000	F-CAGAAGGGATACATATACA R-CAGCCCCTGGCTGTCTCTTGTC	AF223649
TmaA02	247-251	$(CACT)_2(CA)_{16}$	3	0.510	0.541	F-CTCAGTCCAAACAGCTAATG R-TAGTCATTTGTGCAGAGTGC	AF223650
TmaA03	163-183	$(GACA)_4$	2	0.303	0.398	F-ACATGTGTTCCCTGCTGTAT R-GATTTTTGGAGCAGTTGTCA	AF223651
TmaA04	204	$(CT)_2(GT)_{12}AT(GT)_7AT(GT)_2$	1	0.000	0.000	F-GAACACAAGACCGCAATAAC R-TGGTGTATCACTCAGGGTTC	AF223652
TmaA09	150	$(GT)_{15}$	1	0.000	0.000	F-GATGGGATACTGGGTTATGC R-ATGCAGACACTGGACATAGG	AF223653
TmaE02	172-174	$(GT)_{13}$	2	0.440	0.463	F-GTCTCTACGGCCTAGAATTGTG R-TTTCTCTACCTCTCCTCACACG	AF223656
TmaE08	149-165	$(CA)_{13}TA(CA)_5$	3	0.470	0.545	F-GAATAGAGACTGGGCTAGAATCC R-GCCTTTTGGAGGGATAGAAGTAG	AF223657
TmaE11	177-197	$(CA)_{13}$	6	0.583	0.626	F-ACACACAACATCACTCATCCAC R-AAGCTGCGTTCTACTTCATATAATC	AF223658
TmaE26	199-201	$(CA)_8C(CA)_{17}$	2	0.243	0.255	F-CATTCCTGATCCACAAAATC R-CCTGTCTTCTCTCTGTTTCTCC	AF223659
TmaF14	204-206	$(TC)_6(TG)_2TA(TC)_5TG(TC)_3$	2	0.188	0.364	F-CTAAGACATTGCTCCAAAAGC R-GGGCAGTGGGATTTGAGATG	AF223660
TmaF34	271	$(TCTCTCTCTTTCTG)_2(TC)_4TT(TC)_3$ $(AC)_8AT(AC)_9$	1	0.000	0.000	F-CATGAGAGACTATGCTCCCTTC R-CAGGTAGGAAGATGATGAGGAC	AF223661
TmaH11	298	$(TCTG)_4(TCTA)_5CCTGTCTATCCA$ $(TCTA)_3CCTG(TCTA)_8CCTG(TCTA)_5$	1	0.000	0.000	F-AGCAGATAGACACACTGGGAAG R-GAGTCTGAATGAATGAATTACTGC	AF223662
TmaH23	244	$TCTA)_2(TCTG)(TCTA)(TCTG)_2$ $(TCTA)_4(TGTA)(TCTA)_4$	1	0.000	0.000	F-CGTAGGGAACAAATAGCCTTAG R-ACTGCCAACCTTTCCATTATC	AF223663
TmaM61	176	$(TG)_3(GT)_{17}$	1	0.000	0.000	F-TTGAGGTGTAATCTGTGTG R-GGTAATCGGAGTTGGTGTA	AF223655
TmaM79	154-156	$(GT)_{15}$	2	0.561	0.535	F-CCAATCATGTCCCAAACT R-CAATAGAAGAAGCAGCAG	AF223654

Monomorphic loci are included due to their demonstrated (Table 3-2) utility in other Sirenian species. Number of alleles per locus was obtained by screening fifty manatees from throughout the Florida peninsula.

Table 3-2. Cross-species amplification of 15 Florida manatee (*Trichechus manatus latirostris*) microsatellite loci in three Sirenian species: Antillean manatee (*Trichechus manatus manatus*), Amazonian manatee (*Trichechus inunguis*), and the dugong (*Dugong dugong*).

	Antillean Manatee *N*=21		Amazonian manatee *N*=7		Dugong *N*=3	
	Product	Number of Alleles	Product	Number of Alleles	Product	Number of Alleles
TmaA01	+	2	+	2	+	2
TmaA02	+	3	+	5	+	2
TmaA03	+	4	+	3	+	1
TmaA04	+	3	+	1	+	3
TmaA09	+	1	+	4	+	3
TmaE02	+	2	+	1	+	3
TmaE08	+	4	+	2	+	3
TmaE11	+	8	+	1	+	3
TmaE26	+	5	+	3	+	2
TmaF14	+	2	+	2	+	1
TmaF34	+	1	+	2	-	-
TmaH23	+	1	+	1	+	1
TmaH11	+	1	+	3	+	1
TmaM79	+	3	+	3	+	2
TmaA61	+	2	+	2	+	1

N is number of individuals surveyed, "+" indicates the amplified fragment was similar in size to that observed in the Florida manatee, and "-" indicates no or suboptimal amplification products.

CHAPTER 4
POPULATION GENETIC STRUCTURE AND GENE FLOW IN THE FLORIDA MANATEE (*Trichechus manatus latirostris*) INFERRED FROM MICROSATELLITE LOCI

Introduction

The West Indian manatee (*Trichechus manatus*) is a warm-water tropical mammal of the Order Sirenia (Domning & Hayek 1986). Two subspecies are described: the Antillean manatee (*T. m. manatus*), distributed within major river basins and coastal habitats of Central and South America and the Greater Antilles, and the Florida manatee (*T. m. latirostris*), which occurs primarily in Florida and, to a lesser extent, other states of the southeastern U.S. (Lefebvre *et al.* 1989). Their distribution is influenced by temperature and availability of shelter, aquatic vegetation, and fresh water (Lefebvre *et al.* 1989). During the colder months of the year, from November to March, manatees seek sources of warm water to avoid hypothermia (Irvine 1983). Therefore, winter refuges such as natural springs and warm-water discharges from power and paper plants are important factors affecting their seasonal movements and distribution.

Hunting and poaching, as well as human encroachment into manatee habitat, have been ongoing for the last several centuries, which helps to explain the manatee's currently low population levels throughout much of its range (Lefebvre *et al.* 1989). As human populations have increased, freshwater ecosystems are more heavily impacted by recreation and commerce, thereby reducing the availability of natural resources and shelter for the species. The major cause of human-related manatee mortality in Florida is collision with boats and, to a lesser extent, entrapment in gates of locks and dams, and net entanglement (O'Shea *et al.* 1985). In addition, manatees are vulnerable to cold weather (O'Shea *et al.* 1985, Ackerman *et al.* 1995) and red-tide outbreaks (Buergelt *et al.* 1984; O'Shea *et al.* 1991; Bossart *et al.* 1998). In U.S. waters the manatee is listed as an endangered species (U.S. Fish and Wildlife Service 1982) and is protected under the U.S. Marine Mammal Protection Act of 1972. Studies by the U.S. Fish and Wildlife Service, U.S. Geological Survey, Florida Fish and Wildlife Conservation Commission, universities, and other organizations, have provided the scientific rationale for the establishment of manatee sanctuaries and slow speed zones for boats. These regulatory measures are intended to decrease the rate of human-related manatee mortality (U.S. Fish and Wildlife Service 1996). In addition, management plans and local regulations affecting manatee habitat have benefited from studies on life history, patterns of seasonal movements, and distribution (Rathbun *et al.* 1990; O'Shea *et al.* 1995; Deutsch *et al.* 1998).

Genetic studies to determine levels of population structure, gene flow, and genetic diversity within the Florida manatee were proposed by the Florida Manatee Recovery Plan (1995). Results from an allozyme study suggested a moderate-to-low level of genetic variability, with observed heterozygosity of 0.050, and lack of detectable population genetic structure among distinctive geographic regions within Florida (McClenaghan and O'Shea 1988). Low genetic diversity has also been reported in mtDNA studies of cytochrome *b* (Bradley *et al.* 1993) and the control region (Garcia-Rodriguez *et al.* 1998). These results are not surprising as manatees are characterized by loose social bond (Hartman 1979; Deutsch *et al.* 1998), both females and males are capable of long seasonal migration movements, and mixing occurs along both coasts of the Florida peninsula (Deutsch *et al.* 1998). The genetic evidence provided by Garcia-Rodriguez *et al.* (1998) showed strong genetic structure across the range of the West Indian manatee (*T. manatus*), and three provisional Evolutionarily Significant Units (ESU), or unit of conservation below the species level (Waples 1991; Moritz 1994a). In addition, this study revealed only a single haplotype in the Florida samples, indicating that the Florida manatee can be considered a single ESU. This means that the Florida population is unique as demonstrated by "quantitative measures of genetic or morphological discontinuity" (U.S. Department of the Interior et al. 1996). However, it is still unclear if Florida manatees are divided into Management Units (MUs), which are "populations with significant divergence of allele frequencies at nuclear or mitochondrial loci, regardless of the phylogenetic distinctiveness of the alleles" (Moritz 1994a), and if so, under what biogeographic context should those management units be defined?

When the Manatee Salvage Program was initiated in 1974, carcass retrieval was allocated according to the proximity of carcasses to the recovery location (O'Shea *et al.* 1985). Carcasses were identified as recovered from either north or south Florida (Campbell & Irvine 1981). In the 1980's and 90's, management strategies and study plans designed for the protection of manatee populations in Florida were delineated according to geographic regions where manatees aggregated during the winter months (McClenaghan and O'Shea 1988). On peninsular Florida these regions are: the northeast (MNE), the east-central (MEC), the southeast (MSE), the southwest (MSW), and northwest (MNW) (Figure 4-1). Since little information on movement patterns during the warm season or habitat utilization was available then, geographic regions were based on winter aggregations and logistical decisions. McClenaghan & O'Shea (1988) used these regions for their study on manatee genetics to determine if these partitions could have a genetic component as well. Their results rejected the hypothesis of population genetic differences and promoted the alternative hypothesis of extensive gene flow and movement between regions on each coast. It was suggested that the low rate of migration between the east and west coasts occurred due to habitat unsuitability and geographical barriers in the southern Florida peninsula (Moore 1951); however, allozymes did not provide sufficient resolution to test the hypothesis of population differentiation. To explore these questions further, microsatellite markers were used because they have proven useful in detecting fine-scale genetic differentiation on small sized populations with low levels of genetic differentiation (Waldick *et al.* 1999).

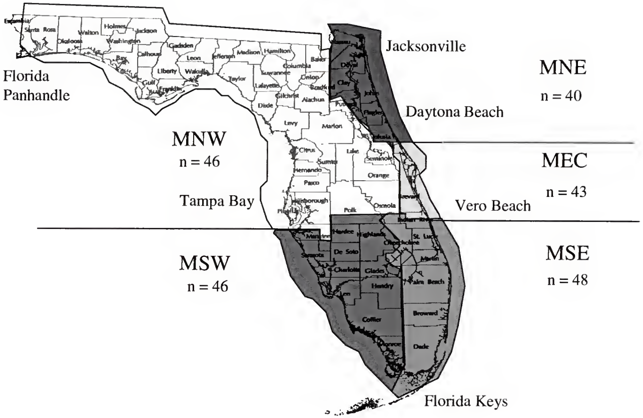


Figure 4-1. Distribution of Florida manatee carcass samples according to the geographic origin of the samples as designated by FFWCC field personnel (O'Shea *et al.* 1995).

In the present study, the variation in microsatellite loci among the five regions described above for the current manatee salvage program in Florida was analyzed to test three hypotheses. The first test was performed to determine if genetic evidence supports differentiation between the manatee populations from the east and west coasts. The second and third tests were performed to see if there is any level of genetic differences among the five regions described by the Florida Fish and Wildlife Conservation Commission (FFWCC) and the Manatee Population Status Working Group (MPSWG). Allele frequency shifts were compared to determine to what degree the populations are genetically related. This study offers a genetic component to migratory information using estimates of gene flow levels and allele distribution for each region.

Materials and Methods

Sample Distribution

Samples for this study were collected from individual manatee carcasses recovered by the FFWCC's Manatee Salvage Program from 1996 to 1999. A total of 223 carcasses was sampled from the east (n = *131*) and west (n = *92*) coasts (see Appendix). Samples from both coasts were further separated into regions based on the geographic origin of the samples as designated by FFWCC field personnel. Three regions were determined along the east coast of Florida: the northeast (MNE), extending from Nassau County to Volusia County and including the St. Johns River (n = *40*); the east-central (MEC), comprising Brevard and Indian River counties (n = *43*); and the southeast (MSE), from St. Lucie County to the Florida Keys (n = *48*). Two regions were determined along the west coast of the peninsula: the southwest (MSW), extending from Monroe County to Sarasota County (n = *46*); and the northwest (MNW), extending from Manatee County through the Florida panhandle (n = *46*) (Figure 4-1). In addition, samples from the east and west coasts were separated into subpopulation regions according to designations proposed by the MPSWG. These regions are: the Atlantic, extending from Nassau County to the Florida Keys and including the Lower St. Johns River (n=*129*); the Upper St. Johns River, extending from about 20 miles north of Palatka in Putman County to the north section of Lake County and including the Upper St. Johns River (*n=2*); the Southwest, extending from Monroe County to Charlotte County (*n=44*); the Central-West, extending from Sarasota County to Pasco County (*n=37*); and the Northwest, extending from Hernando County to the Florida panhandle (*n=11*) (Figure 4-2). Three sample treatments were performed in this study to determine the level of population genetic structure among designated regions. In the first one, two populations of manatees that are considered to be geographically distant and separated (but not isolated) by the peninsula of Florida (east vs. west) were used. In the second treatment, populations were separated on each coast into the geographically distinctive regions as designated by FFWCC (MNE, MEC, MSE, MSW and MNW). In the third treatment, populations were separated on each coast into geographically distinctive regions as designated by the Manatee Population Status Working Group (Atlantic, Upper St. Johns River, Southwest, Central-West, and Northwest).

DNA Isolation

Tissue samples, mostly from skin and muscle, were collected during necropsy examination and stored in a high concentration salt buffer (250 mM EDTA, pH 7.5, 20% DMSO, and saturated salt). Extraction of DNA was performed following the isolation protocol from Gentra's Puregene™ Isolation Kit. Briefly, approximately 10-20 mg of skin or muscle sample were minced and added to a lysis buffer containing an anionic detergent that solubilizes the cellular components. Proteinase K solution (20 mg/ml) was added to the samples and incubated at 65°C overnight. In addition, a protein precipitation solution was included to eliminate cytoplasmic and nuclear proteins. Genomic DNA was then precipitated with 100% isopropanol, washed with 70% ethanol, dried in a vacuum microcentrifuge, and resuspended in TE buffer (Tris-HCl, pH 8.0; EDTA, pH 8.0). DNA was quantified using a fluorometer and dilutions of approximately 100 ng/µl were used to prepared a template for PCR amplification.

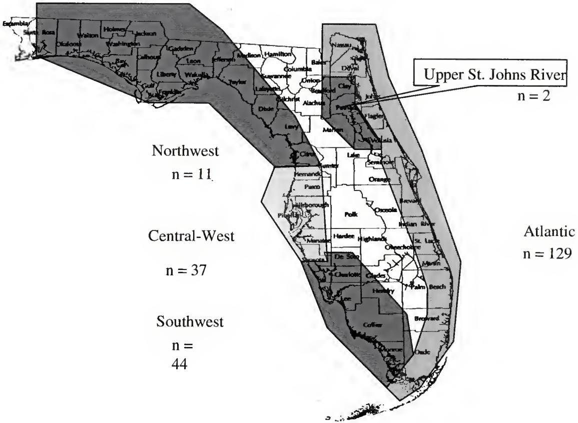


Figure 4-2. Geographic regions based on wintering sites as proposed by the Manatee Population Status Working Group.

Microsatellite Loci

PCR reactions were optimized to work under multiplexing conditions by combining several pairs of primers in a PCR reaction tube. Approximately 200 ng of genomic DNA were used in a 20 µl volume reaction containing 5X multiplexing buffer (83 mM $(NH_4)_2SO_4$, 335 mM Tris-HCl pH 8.3, 50 mM ß-mercaptoethanol, 34 mM EDTA), 0.3175 mM of each dNTP, 0.275 µM of each primer, and 0.08 units of Taq DNA polymerase (Gibco BRL). Eight microsatellite loci, with either di- or tetramer repeats, specifically designed for the Florida manatee (TmaA02, TmaA03, TmaE02, TmaE08, TmaE11, TmaE26, TmaF14, and TmaM79) were amplified following PCR conditions described in Garcia-Rodriguez *et al.* (*in press*). Samples were run on an ABI PRISM™ 310 Genetic Analyzer (Perkin Elmer) and alleles were analyzed and scored using the ABI PRISM™ Genotyper® 2.0 software (Perkin Elmer).

Statistical Analysis

At the preliminary stage of the data analysis using 223 samples there was concern that missing data (missing alleles) could have an effect on the overall results in testing the three hypotheses. To explore this further a qualitative exercise was performed where all of the samples ($n = 87$) that had more than two missing loci were removed and only those individuals ($n = 136$) that were successfully genotyped for seven or eight loci were considered for analysis. To simplify the results and discussion sections, the different treatments were labeled as follows: treatment A corresponds to the analysis of east versus west coast using 223 samples; treatment B corresponds to the analysis of east versus west coast using 136 individuals; treatment C is the comparison of the 5 regions as proposed by FFWCC using 223 samples; treatment D is the analysis of the 5 regions as proposed by the FFWCC using 136 samples; treatment E corresponds to the analysis of the 5 regions proposed by the MPSWG using 223 samples; and finally, treatment F is the analysis of the sites as proposed by the MPSWG using 136 samples.

Population genetic structure

Three different approaches were used to test for population genetic structure in sample treatments A through D. (1) The allele frequency differences among populations of manatees were tested using the Fisher's exact test (Fisher 1970) where an unbiased estimate of the P-value of the probability test is obtained. The null hypothesis (Ho) "the allelic distribution is uniform across populations" was tested for each locus on a contingency table. (2) The differences in genotypic distribution across the populations were based on estimates of Wright's F-statistics (Wright 1951) which applies Weir and Cockerham's unbiased estimator (θ) of F-statistics (Weir & Cockerham 1984; Weir 1990). The null hypothesis, $F_{st} = 0$ "the genotypic distribution is uniform across populations" was tested for all loci using a chi-square goodness-of-fit test (Workman & Niswander, 1970) performed to evaluate the significance of the values obtained: $\chi^2 = 2NF_{st}(k - 1)$; $d.f. = (k - 1)(s - 1)$, where N is the total number of individuals sampled, k is the number of loci used for the analysis, and s the number of populations. (3) The estimate of Rho_{st} statistics is a measure analogous to F_{st} but incorporates allele sizes estimates. This estimator is equivalent to Slatkin's R_{st} (Slatkin 1995) and it assumes a step-wise mutation model. Calculations for the three estimators were performed using GENEPOP Version 1.2 (Weir 1990; Raymond & Rousett 1995; Michalakis & Excoffier 1996). These analyses were not performed for treatments E and F, first because of the small sample size for the St. Johns River region; removing this region from the analysis would be similar to testing for population differentiation between the east and the west coast regions. Second, tests performed in the previous treatments were enough to determine population structure among the regions within each coast. Third, results on the assignment tests (described below) performed for these two treatments supported the decision.

An assignment test by Paetkau *et al.* (1995) was performed on all the sample treatments. This test assigns individuals to the different sampling areas by first calculating the expected frequency of each individual's genotype in each population and then assigning each individual to the population in which its genotype is expected to have the highest frequency. The calculation is based on expected genotype frequency at each of the eight loci. This calculation assumes random mating and linkage equilibrium within each population.

Deviation from Hardy-Weinberg Equilibrium

GENEPOP was used to calculate the exact test for fit of genotype proportions to Hardy-Weinberg Equilibrium (HWE) (Guo & Thompson 1992). Overall heterozygote deficiencies within populations and estimates of observed heterozygosity (H_O) and expected heterozygosity (H_E) values for the populations were calculated according to Nei (1987). The unbiased expected heterozygosity at each locus in every population was estimated as $H_E = 2n(1 - \sum pi^2)/(2n-1)$, where pi is the frequency of each of the alleles at a locus and n is the number of individuals sampled (Nei 1987; p178, eqn. 84). Significant deviation from the Hardy-Weinberg expectations was measured by the inbreeding index, F_{is}, calculated for each locus according to Nei (1987) using BIOSYS version 1.7 (Swofford 1989). The null hypothesis: "random mating" $F_{is} = 0$, was evaluated by a chi-square goodness-of-fit test (Li & Horvitz, 1953):

$\chi^2 = F_{is}2N(k-1)$; $d.f. = k(k-1)/2$, where N is the number of individuals in the population and k the number of alleles in the locus tested.

Gene flow estimates

Gene flow estimates were calculated using GENEPOP from the approximation of F_{st} ($1/(1+4Nm)$) in Wright's infinite island model, when $m \ll 1$ and is expressed in terms of N_em (effective number of migrants per generation) (Wright 1951). In addition, gene flow estimates were obtained with Rho_{st} statistics, where $Nm = (d_s - 1/4d_s)(1/Rho - 1)$ and d_s is the number of populations. These statistics are based on assumptions of a stepwise mutation model (Slatkin 1995; Michalakis & Excoffier 1996). Both models agree in that they take into consideration drift as one of the evolutionary forces. The major difference between them is that F_{st} takes into consideration gene flow, whereas Rho_{st} considers the mutational history of the alleles. Finally, a multilocus estimate of the effective number of migrants (*Nm*), based on the private allele approach, was performed (Slatkin 1985a; Slatkin & Barton 1989).

Genetic distance

The genetic divergence between the five defined manatee regions was calculated with Cavalli-Sforza and Edwards' (1967) chord distance (D_{CE}) after 2,000 bootstrap replicates, and used to construct a population tree with the NJBPE2 computer program. This method has a greater probability of representing the correct tree topology among related populations that may follow either the infinite alleles or stepwise mutation model (Nei & Takezaki 1996; Takezaki & Nei 1996). In addition, this method does not make any assumptions regarding constant population size or mutation rates among loci.

A pairwise genetic distance among all the individuals used in this study was determined using the proportion of shared allele distance (D_{ps}) according to the software package MICROSAT (Minch *et al.* 1995).

Results

Polymorphism and Genetic Variability in the Florida Manatee Population

Eight polymorphic DNA microsatellite loci were identified for the Florida manatee. Overall, the number of alleles detected in this survey was 23. In general, for treatments A and B, the same number of alleles ($n = 23$) was detected in the east coast population. For the west coast population, 21 alleles were found in treatment A and 20 alleles in treatment B (Tables 4-1 and 4-2). The number of alleles ($n= 21$ and $n = 23$) remained unchanged for region MNE and MNW in treatments C and D (Tables 4-3 and 4-4). In contrast, more alleles were found for regions MEC ($n = 22$), MSE ($n = 20$) and MSW ($n = 19$) for treatment C. The number of alleles detected

Table 4-1. Allele frequencies of microsatellite loci for treatment A ($N = 223$).

	TmaE02			TmaE08			TmaE11			TmaE26	
	EAST	WEST		EAST	WEST		EAST	WEST		EAST	WEST
	($n = 99$)	($n = 73$)		($n = 105$)	($n = 76$)		($n = 102$)	($n = 66$)		($n = 108$)	($n = 70$)
1	0.318	0.425	1	0.324	0.434	1	0.505	0.614	1	0.792	0.936
2	0.682	0.575	2	0.105	0.026	2	0.005	0.000	2	0.208	0.064
			3	0.571	0.539	3	0.054	0.000			
						4	0.255	0.227			
						5	0.078	0.144			
						6	0.103	0.015			
A*	2	2		3	3		6	4		2	2

	TmaF14			TmaA02			TmaA03			TmaM79	
	EAST	WEST		EAST	WEST		EAST	WEST		EAST	WEST
	($n = 74$)	($n =17$)		($n = 116$)	($n = 89$)		($n = 110$)	($n = 86$)		($n = 118$)	($n = 79$)
1	0.250	0.147	1	0.203	0.331	1	0.305	0.233	1	0.547	0.525
2	0.750	0.853	2	0.573	0.663	2	0.695	0.767	2	0.411	0.424
			3	0.224	0.006				3	0.042	0.051
A*	2	2		3	3		2	2		3	3

A* = number of alleles

Table 4-2. Allele frequencies of microsatellite loci for treatment B ($N = 136$).

	TmaE02			TmaE08			TmaE11			TmaE26	
	EAST	WEST		EAST	WEST		EAST	WEST		EAST	WEST
	($n = 73$)	($n = 55$)		($n = 80$)	($n = 56$)		($n = 80$)	($n = 53$)		($n = 79$)	($n = 56$)
1	0.349	0.427	1	0.281	0.438	1	0.504	0.604	1	0.785	0.937
2	0.651	0.573	2	0.106	0.026	2	0.006	0.000	2	0.215	0.063
			3	0.613	0.536	3	0.054	0.000			
						4	0.250	0.226			
						5	0.062	0.151			
						6	0.124	0.019			
A*	2	2		3	3		6	4		2	2

	TmaF14			TmaA02			TmaA03			TmaM79	
	EAST	WEST		EAST	WEST		EAST	WEST		EAST	WEST
	($n = 57$)	($n = 8$)		($n = 79$)	($n = 56$)		($n = 78$)	($n = 55$)		($n = 80$)	($n = 55$)
1	0.272	0.063	1	0.171	0.286	1	0.276	0.164	1	0.556	0.527
2	0.728	0.937	2	0.551	0.714	2	0.724	0.836	2	0.394	0.400
			3	0.278	0.000				3	0.050	0.073
A*	2	2		3	2		2	2		3	3

A* = number of alleles

Table 4-3. Allele frequencies of microsatellite loci for treatment C ($N = 223$).

	TmaE02						TmaE08				
	MEC	MNE	MNW	MSE	MSW		MEC	MNE	MNW	MSE	MSW
	($n = 43$)	($n = 40$)	($n = 46$)	($n = 48$)	($n = 46$)		($n = 43$)	($n = 40$)	($n = 46$)	($n = 48$)	($n = 46$)
1	0.328	0.222	0.405	0.375	0.444	1	0.264	0.426	0.500	0.310	0.364
2	0.672	0.778	0.595	0.625	0.556	2	0.097	0.074	0.038	0.130	0.014
						3	0.639	0.500	0.462	0.560	0.622
A*	2	2	2	2	2		3	3	3	3	3

	TmaE11						TmaE26				
	MEC	MNE	MNW	MSE	MSW		MEC	MNE	MNW	MSE	MSW
	($n = 43$)	($n = 40$)	($n = 46$)	($n = 48$)	($n = 46$)		($n = 43$)	($n = 40$)	($n = 46$)	($n = 48$)	($n = 46$)
1	0.500	0.400	0.671	0.575	0.529	1	0.708	0.742	0.927	0.910	0.948
2	0.000	0.000	0.000	0.013	0.000	2	0.292	0.258	0.073	0.090	0.052
3	0.043	0.109	0.000	0.025	0.000						
4	0.329	0.273	0.195	0.175	0.275						
5	0.057	0.109	0.110	0.087	0.196						
6	0.071	0.109	0.024	0.125	0.000						
A*	5	5	4	6	3		2	2	2	2	2

Table 4-3—continued.

	TmaF14						TmaA02				
	MEC	MNE	MNW	MSE	MSW		MEC	MNE	MNW	MSE	MSW
	($n = 43$)	($n = 40$)	($n = 46$)	($n = 48$)	($n = 46$)		($n = 43$)	($n = 40$)	($n = 46$)	($n = 48$)	($n = 46$)
1	0.130	0.357	0.125	0.267	0.154	1	0.270	0.214	0.322	0.136	0.341
2	0.870	0.643	0.875	0.733	0.846	2	0.703	0.457	0.667	0.557	0.659
						3	0.027	0.329	0.011	0.307	0.000
A*	2	2	2	2	2		3	3	3	3	2

	TmaA03						TmaM79				
	MEC	MNE	MNW	MSE	MSW		MEC	MNE	MNW	MSE	MSW
	($n = 43$)	($n = 40$)	($n = 46$)	($n = 48$)	($n = 46$)		($n = 43$)	($n = 40$)	($n = 46$)	($n = 48$)	($n = 46$)
1	0.329	0.293	0.326	0.293	0.140	1	0.592	0.559	0.577	0.500	0.475
2	0.671	0.707	0.674	0.707	0.860	2	0.382	0.441	0.423	0.413	0.415
						3	0.026	0.000	0.000	0.087	0.110
A*	2	2	2	2	2		3	2	2	3	3

A* = number of alleles

Table 4-4. Allele frequencies of the microsatellite loci for treatment D ($N = 136$).

	TmaE02						TmaE08				
	MEC	MNE	MNW	MSE	MSW		MEC	MNE	MNW	MSE	MSW
	(n = 20)	(n = 20)	(n =33)	(n = 33)	(n = 22)		(n = 24)	(n = 21)	(n = 34)	(n =35)	(n = 22)
1	0.450	0.225	0.409	0.364	0.455	1	0.250	0.357	0.485	0.257	0.364
2	0.550	0.775	0.591	0.636	0.545	2	0.104	0.095	0.044	0.114	0.000
						3	0.646	0.548	0.471	0.629	0.636
A*	2	2	2	2	2		3	3	3	3	2

	TmaE11						TmaE26				
	MEC	MNE	MNW	MSE	MSW		MEC	MNE	MNW	MSE	MSW
	(n = 24)	(n = 21)	(n = 34)	(n =35)	(n = 19)		(n = 24)	(n = 21)	(n = 34)	(n =34)	(n =22)
1	0.500	0.429	0.647	0.557	0.526	1	0.667	0.738	0.912	0.897	0.977
2	0.000	0.000	0.000	0.014	0.000	2	0.333	0.262	0.088	0.103	0.023
3	0.063	0.071	0.000	0.029	0.000						
4	0.292	0.286	0.191	0.200	0.289						
5	0.042	0.071	0.133	0.071	0.185						
6	0.103	0.143	0.029	0.129	0.000						
A*	5	5	4	6	3		2	2	2	2	2

Table 4-4—continued.

	TmaF14						TmaA02				
	MEC	MNE	MNW	MSE	MSW		MEC	MNE	MNW	MSE	MSW
	(n = 15)	(n =17)	(n = 4)	(n = 25)	(n = 4)		(n = 24)	(n = 21)	(n = 34)	(n = 34)	(n =22)
1	0.133	0.382	0.125	0.280	0.000	1	0.333	0.095	0.324	0.103	0.227
2	0.867	0.618	0.875	0.720	1.000	2	0.625	0.500	0.676	0.529	0.773
						3	0.042	0.405	0.000	0.368	0.000
A*	2	2	2	2	1		3	3	2	3	2

	TmaA03						TmaM79				
	MEC	MNE	MNW	MSE	MSW		MEC	MNE	MNW	MSE	MSW
	(n = 23)	(n = 20)	(n = 33)	(n = 35)	(n = 22)		(n = 24)	(n = 21)	(n = 33)	(n = 35)	(n = 22)
1	0.239	0.275	0.227	0.300	0.068	1	0.625	0.548	0.606	0.514	0.409
2	0.761	0.725	0.773	0.700	0.932	2	0.375	0.452	0.394	0.372	0.409
						3	0.000	0.000	0.000	0.114	0.182
A*	2	2	2	2	2		2	2	2	3	3

A* = number of alleles

per locus was in general very low, ranging from two (TmaE02, TmaE26, TmaF14 and TmaA03) and three alleles (TmaE08, TmaA02, TmaM79) to a maximum of six alleles (TmaE11). The low numbers of alleles per locus detected overall might not be sufficient for paternity analysis. However, the overall number of alleles (19 to 23) in each region might compensate for this low level of allelic diversity and some power (although not considerable) might be sufficient to shed information on population genetic structure.

A second measure of genetic diversity was made by determining the levels of heterozygosity in each population (see Table 4-5). For all the treatments, the levels of differences between the observed and the expected values of heterozygosity was non-significant based on a chi-square goodness-of-fit test. Mean observed heterozygosity (direct count) was higher for the east coast population in both treatments A and B in comparison with the population from the west coast. Region MNE had the highest level of observed heterozygosity for treatment C, while region MEC had the highest for treatment D. Region MSW presented the lowest levels of mean observed heterozygosity, for both treatments C and D.

The Hardy-Weinberg exact test results (Table 4-6) suggested that the east coast population was out of HWE under treatment A for loci TmaM79 ($P = 0.0033$), TmaA02 ($P = 0.000$), TmaF14 ($P = 0.0105$) and TmaA03 ($P = 0.0123$). For treatment B (Table 4-7), all of the loci mentioned above were out of equilibrium ($0.01 < P < 0.5$) for the east coast, except for locus TmaM79. The results for the west coast population suggested that it was out of HWE for locus TmaM79 ($P = 0.0005$) in both treatments A and B.

For treatments C and D, regions MNW and MSE were in Hardy-Weinberg proportions for all 8 loci (Table 4-8 and 4-9). For region MNE, locus TmaA02 ($P = 0.0001$) was out of HWE only in treatment C. Finally, region MEC was out of HWE for locus TmaA02 ($P = 0.0055$) in treatment C, and locus TmaF14 ($P = 0.0038$) in treatment D. Region MSW was out of HWE for locus TmaE08 ($P = 0.0015$ and $P = 0.0054$) in both treatments and only out of equilibrium for

Table 4-5. Mean heterozygosity and mean F_{is} statistics.

		Mean Heterozygosity			Mean	
Population	Treatment	Ho	He	χ^2 *(d.f.)*	F_{is}	χ^2 *(d.f.)*
EAST	A	0.427	0.489	0.0079 (7)	0.1280	15.02 (28)
	B	0.429	0.491	0.0078 (7)	0.1286	9.26 (28)
WEST	A	0.381	0.413	0.0025 (7)	0.0979	6.17 (28)
	B	0.366	0.385	0.009 (7)	0.0464	0.84 (28)

		Mean Heterozygosity			Mean	
Population	Treatment	Ho	He	χ^2 *(d.f.)*	F_{is}	χ^2 *(d.f.)*
MEC	C	0.417	0.457	0.0035 (7)	0.1068	3.43
	D	0.437	0.468	0.0021 (7)	0.0984	1.62
MNE	C	0.451	0.511	0.0070 (7)	0.1045	3.05
	D	0.423	0.506	0.0136 (7)	0.1431	3.01
MNW	C	0.396	0.415	0.0009 (7)	0.055	0.89
	D	0.404	0.409	0.0001 (7)	0.005	1.3
MSE	C	0.414	0.478	0.0086 (7)	0.1154	4.47
	D	0.421	0.480	0.0073 (7)	0.1051	2.7
MSW	C	0.377	0.409	0.0025 (7)	0.0641	1.32
	D	0.318	0.348	0.0026 (7)	0.1141	2

treatment A = 2 pop, 223; treatment B = 2 pop, 136; treatment C = 5 pop, 223; treatment D = 5 pop, 136.

Table 4-6. Observed (*Ho*) and expected (*He*) heterozygosities, allele number (*A*) and test for goodness of fit to the Hardy-Weinberg expectations performed by testing the two alternative hypotheses—heterozygote deficit and heterozygote excess in the east and west coasts. (Treatment A, $N = 223$).

	TmaE02						TmaE08						TmaE11					
	Ho	*He*	*A*	F_{is}	χ^2	*d.f.*	*Ho*	*He*	*A*	F_{is}	χ^2	*d.f.*	*Ho*	*He*	*A*	F_{is}	χ^2	*d.f.*
EAST	0.354	0.436	2	0.185*	4.48	1	0.467	0.560	3	0.163	6.96	3	0.608	0.664	6	0.080	4.19	15
WEST	0.493	0.492	2	-0.009	0.01	1	0.382	0.523	3	0.266*	13.01	3	0.530	0.555	4	0.037	0.37	6

	TmaE26						TmaF14						TmaA02					
	Ho	*He*	*A*	F_{is}	χ^2	*d.f.*	*Ho*	*He*	*A*	F_{is}	χ^2	*d.f.*	*Ho*	*He*	*A*	F_{is}	χ^2	*d.f.*
EAST	0.343	0.331	2	-0.039	0.20	1	**0.257**	**0.378**	2	0.315*	12.99	1	**0.526**	**0.583**	3	0.093	2.26	3
WEST	0.100	0.121	2	0.169	2.62	1	0.176	0.258	2	0.297*	8.11	1	0.494	0.453	3	-0.097	1.73	3

	TmaA03						TmaM79					
	Ho	*He*	*A*	F_{is}	χ^2	*d.f.*	*Ho*	*He*	*A*	F_{is}	χ^2	*d.f.*
EAST	**0.318**	**0.426**	2	0.249*	8.12	1	**0.542**	**0.533**	3	-0.022	0.13	3
WEST	0.279	0.359	2	0.218*	4.37	1	**0.595**	**0.545**	3	-0.098	1.76	3

N for east population = 131

N for west population = 92

Bold numbers indicate out of HWE. Gray area indicates heterozygote deficiency

* indicate F_{is} values that are significantly different from zero ($P<0.05$)

Table 4-7. Observed (*Ho*) and expected (*He*) heterozygosities, allele number (*A*) and test for goodness of fit to the Hardy-Weinberg expectations performed by testing the two alternative hypotheses—heterozygote deficit and heterozygote excess in the east and west coast populations. (Treatment B, $N = 136$).

	TmaE02						TmaE08						TmaE11					
	Ho	*He*	*A*	F_{is}	χ^2	*d.f.*	*Ho*	*He*	*A*	F_{is}	χ^2	*d.f.*	*Ho*	*He*	*A*	F_{is}	χ^2	*d.f.*
EAST	0.397	0.458	2	0.126	1.27	1	0.500	0.538	3	0.064	0.65	3	0.613	0.663	6	0.071	25.00	15
WEST	0.527	0.494	2	-0.077	0.33	1	0.393	0.526	3	0.246	6.77	3	0.566	0.566	4	-0.009	12.59	6

	TmaE26						TmaF14						TmaA02					
	Ho	*He*	*A*	F_{is}	χ^2	*d.f.*	*Ho*	*He*	*A*	F_{is}	χ^2	*d.f.*	*Ho*	*He*	*A*	F_{is}	χ^2	*d.f.*
EAST	0.354	0.34	2	-0.049	0.19	1	**0.228**	**0.399**	2	0.424*	14.38	1	**0.494**	**0.594**	3	0.163	4.25	3
WEST	0.089	0.118	2	0.238	3.17	1	0.125	0.125	2	-0.067	0.25	1	0.429	0.412	2	-0.050	0.14	1

	TmaA03						TmaM79					
	Ho	*He*	*A*	F_{is}	χ^2	*d.f.*	*Ho*	*He*	*A*	F_{is}	χ^2	*d.f.*
EAST	**0.295**	**0.402**	3	0.262*	10.98	3	0.550	0.536	3	-0.032	0.16	3
WEST	0.255	0.276	2	0.070	0.54	1	**0.545**	**0.562**	3	0.020	0.04	3

Bold numbers indicate out of HWE. Gray area indicates heterozygote deficiency
* indicate F_{is} values that are significantly different from zero ($P<0.05$)

Table 4-8. Observed (*Ho*) and expected (*He*) heterozygosities, allele number (*A*) and test for goodness of fit to the Hardy-Weinberg expectations performed by testing the two alternative hypotheses, heterozygote deficit and heterozygote excess in the five regions, northeast (MNE), east central (MEC), southeast (MSE), southwest (MSW) and northwest (MNW). (Treatment C, $N = 223$).

	TmaE02						TmaE08						TmaE11					
	Ho	*He*	*A*	F_{is}	χ^2	*d.f.*	*Ho*	*He*	*A*	F_{is}	χ^2	*d.f.*	*Ho*	*He*	*A*	F_{is}	χ^2	*d.f.*
MNE	0.296	0.352	2	0.143	0.81	1	0.444	0.574	3	0.211	3.56	3	0.704	0.737	5	0.027	0.12	10
MEC	0.344	0.448	2	0.220	2.08	1	0.500	0.520	3	0.025	0.05	3	0.571	0.641	5	0.096	1.58	10
MSE	0.400	0.475	2	0.147	1.03	1	0.452	0.581	3	0.212	4.31	3	0.575	0.622	6	0.065	1.01	15
MSW	0.444	0.501	2	0.100	0.46	1	**0.243**	**0.487**	3	0.494*	22.45	3	0.640	0.624	3	-0.047	0.20	3
MNW	0.541	0.489	2	-0.012	0.01	1	0.513	0.542	3	0.042	0.16	3	0.463	0.506	4	0.072	0.71	6

	TmaE26						TmaF14						TmaA02					
	Ho	*He*	*A*	F_{is}	χ^2	*d.f.*	*Ho*	*He*	*A*	F_{is}	χ^2	*d.f.*	*Ho*	*He*	*A*	F_{is}	χ^2	*d.f.*
MNE	0.394	0.388	2	-0.030	0.03	1	0.429	0.47	2	0.067	0.18	1	**0.514**	**0.646**	3	0.193	2.98	3
MEC	0.472	0.419	2	-0.143	0.89	1	0.087	0.232	2	0.617*	16.36	1	**0.486**	**0.438**	3	-0.125	1.34	3
MSE	0.179	0.166	2	-0.099	0.47	1	0.267	0.398	2	0.318*	4.85	1	0.568	0.584	3	0.016	0.02	3
MSW	0.103	0.100	2	-0.055	0.14	1	0.154	0.271	2	0.409*	7.69	1	0.500	0.455	2	-0.113	0.58	1
MNW	0.980	0.137	2	0.281	3.63	1	0.250	0.250	2	-0.143	0.94	1	0.489	0.457	3	-0.083	0.63	3

	TmaA03						TmaM79					
	Ho	*He*	*A*	F_{is}	χ^2	*d.f.*	*Ho*	*He*	*A*	F_{is}	χ^2	*d.f.*
MNE	0.241	4.220	2	0.418*	6.98	1	0.588	0.500	2	-0.193	1.49	1
MEC	0.371	0.448	2	0.158	1.07	1	0.500	0.510	2	0.006	0.00	3
MSE	0.326	0.419	2	0.214	2.19	1	0.543	0.578	3	0.050	0.24	3
MSW	0.279	0.243	2	-0.162	1.20	1	**0.650**	**0.591**	3	-0.113	1.17	3
MNW	0.279	0.444	2	0.365*	6.12	1	0.538	0.495	2	-0.103	0.48	1

Bold numbers indicate out of HWE. Gray area indicates heterozygote deficiency
* indicate F_{is} values that are significantly different from zero ($P<0.05$)

Table 4-9. Observed (*Ho*) and expected (*He*) heterozygosities, allele number (*A*) and test for goodness of fit to the Hardy-Weinberg expectations performed by testing the two alternative hypotheses, heterozygote deficit and heterozygote excess in the five regions, northeast (MNE), east central (MEC), southeast (MSE), southwest (MSW) and northwest (MNW). (Treatment D, $N = 136$)

	TmaE02						TmaE08						TmaE11					
	Ho	*He*	*A*	F_{is}	χ^2	*d.f.*	*Ho*	*He*	*A*	F_{is}	χ^2	*d.f.*	*Ho*	*He*	*A*	F_{is}	χ^2	*d.f.*
MNE	0.350	0.358	2	-0.004	0.00	1	0.476	0.577	3	0.155	1.00	3	0.714	0.721	5	-0.014	0.02	10
MEC	0.400	0.508	2	0.192	0.88	1	0.542	0.520	3	-0.063	0.19	3	0.583	0.662	5	0.100	0.96	10
MSE	0.424	0.470	2	0.083	0.24	1	0.486	0.533	3	0.076	0.40	3	0.571	0.636	6	0.089	1.38	15
MSW	0.545	0.507	2	-0.100	0.22	1	**0.182**	**0.474**	2	0.607*	8.10	1	0.632	0.622	3	-0.043	0.08	3
MNW	0.515	0.491	2	-0.066	0.14	1	0.529	0.549	3	0.022	0.03	3	0.529	0.534	4	-0.006	0.00	6

	TmaE26						TmaF14						TmaA02					
	Ho	*He*	*A*	F_{is}	χ^2	*d.f.*	*Ho*	*He*	*A*	F_{is}	χ^2	*d.f.*	*Ho*	*He*	*A*	F_{is}	χ^2	*d.f.*
MNE	0.429	0.396	2	-0.109	0.24	1	0.412	0.487	2	0.128	0.34	1	**0.333**	**0.591**	2	0.422	3.73	1
MEC	0.500	0.454	2	-0.125	0.37	1	**0.000**	**0.239**	2	1*	24.00	1	**0.583**	**0.507**	3	-0.175	1.47	3
MSE	0.206	0.187	2	-0.115	0.43	1	0.240	0.411	2	0.405*	5.41	1	0.529	0.583	3	0.078	0.40	3
MSW	0.045	0.045	2	-0.023	0.01	1	0.000	0.000	1	0.000	0.00	0	0.455	0.359	2	-0.294	1.90	1
MNW	0.118	0.163	2	0.269	2.46	1	0.250	0.250	2	-0.143	0.69	1	0.412	0.444	2	0.059	0.11	1

	TmaA03						TmaM79					
	Ho	*He*	*A*	F_{is}	χ^2	*d.f.*	*Ho*	*He*	*A*	F_{is}	χ^2	*d.f.*
MNE	**0.150**	**0.409**	2	0.624*	8.17	1	0.524	0.508	2	-0.057	0.06	1
MEC	0.391	0.372	2	-0.075	0.13	1	0.500	0.479	2	-0.067	1.60	1
MSE	0.314	0.426	2	0.252	2.09	1	0.600	0.593	3	-0.027	0.04	3
MSW	0.136	0.130	2	-0.073	0.11	1	0.545	0.647	3	0.137	0.82	3
MNW	0.333	0.357	2	0.051	0.08	1	0.545	0.485	2	-0.142	0.68	1

Bold numbers indicate out of HWE. Gray area indicates heterozygote deficiency

* indicate F_{is} values that are significantly different from zero ($P<0.05$)

locus TmaM79 ($P = 0.0024$) for treatment C. Probability analysis to test for heterozygote deficit at the locus level revealed heterozygote deficit at locus TamE08 for region MSW. At the multilocus level (global test), loci TmaF14 and TmaA03 revealed heterozygote deficit for regions MEC and MSE.

Population Genetic Structure

To determine the population structure of the Florida manatee population, several analyses were performed:

Allele frequency differences – Fisher's test

Pairwise comparisons were performed among all the regions to determine the distribution of alleles as a measure of population differentiation. Allele frequencies obtained for each of the eight loci across the east and west coast manatee populations, and across the five regions (multilocus pairwise comparison), are given in tables 4-1 to 4-4. For treatments A and B, 8 pairwise comparisons (one per locus) were performed between the east and the west coast. For treatments C and D, 80 pairwise comparisons were performed (since there are 5 regions, this generates 10 comparisons per locus and there are 8 loci, which totals 80 comparisons). For treatment A, allele frequencies at loci TmaE02, TmaE26, TmaF14, TmaA03, TmaM79 (or 62.5% of the comparisons of allelic frequencies) were significantly different ($P<0.025$). For treatment B, allele frequencies at loci TmaE02, TmaF14, TmaA03, TmaM79 (or 50%) were significantly different ($P<0.025$) indicating differentiation between the east and the west coast samples. For treatment C, only loci TmaE02 and TmaF14 supported the hypothesis of population genetic differentiation among the regions (P<0.01). In general, from the 80 comparisons performed, 62 of them (77.5%) supported significant genetic differentiation when tested for pairwise comparisons for loci TmaE08 (90%), TmaE11 (70%), TmaE26 (50%), TmaA03 (80%) and TmaM79 (90%). Region MNE was indistinguishable from MNW and MSW based on allele frequency comparisons for loci TmaE11, TmaE26 and TmaA02. No differentiation was detected when comparing region MEC with MNW (TmaE08, TmaE26), MSE (TmaA02, TmaE26), MSW (TmaA03, TmaE26) and MNE (TmaA02). Likewise, region MSE was indistinguishable from MSW (TmaE11, TmaA02) and MNW (TmaA02). Finally, it was not possible to distinguish region MSW from MNW with loci TmaA03 and TmaM79. In general, only the locus TmaA02 provided less than 50% support for the hypothesis of population structure. For treatment D, 83.75% of the comparisons (loci TmaE02, TmaE08 and TmaF14) supported population differentiation, while 40 and 60% of the comparisons performed with loci TmaE26 and TmaA02 respectively could not distinguish populations (P<0.01).

Genotype frequency shifts – F_{st} statistics

Genotypic differentiation between the east and west coasts and among the five regions was tested for each of the eight loci. The null hypothesis (Ho) tested was: "the genotypic distribution is uniform across populations". For each locus, the test was performed on a contingency table and an unbiased estimate of the P-value of an F_{st}-based exact test was performed. Frequency shifts between the east and west populations were evident for loci TmaE02, TmaF14, TmaA03 and TmaM79 (P<0.025) for both treatments A and B, suggesting possible population structure. Frequency shifts at the genotypic level among the five regions was evident for loci TmaE02, TmaE08, TmaF14, TmaM79 and TmaA03 (except when comparing MSW and MEC, where the differences in genotypic frequencies were not statistically significant, P <0.01). In general the results were similar for treatments C and D. The differences in genotype frequencies were not significant in some comparisons for loci TmaE11, TmaE26 and TmaA02 (P<0.01). No population resolution was obtained when comparing region MNE with MNW (TmaE11, TmaE26 and TmaA02) (P<0.01), MSW (TmaE26, TmaA02) (P<0.01) and MEC (TmaA02) (P<0.01). Similarly, no population differentiation was observed when comparing MSE with MEC (TmaE26, TmaA02) (P<0.01), MSW (TmaA02) (P<0.01) and MNW (TmaA02) (P<0.01).

Finally, population structure was unresolved when comparing region MEC with MNW (TmaE26) (*P*<0.01) and MSW (TmaA03, TmaE26) (*P*<0.01).

Assignment test

Results from the multilocus assignment tests performed on the different treatments are summarized in tables 4-10 through 4-12, and figures 4-3 through 4-8. The results obtained from pooling populations into east and west coasts of Florida showed that in treatment A, from 131 individuals sampled on the east coast, 65% were correctly assigned to that area. Likewise, from 92 individuals sampled on the west coast, 87% were correctly assigned. In treatment B, 77% of 81 individuals sampled on the east coast were correctly assigned. Similarly, from 55 individuals sampled on the west coast, 80% were correctly assigned to that area (Table 4-10). For treatments C and D (Table 4-11), the highest percentage values correspond, in the majority of the cases, to correctly assigned individuals; with the second highest percentage corresponding to individuals misassigned to the nearest region within the same coast. In addition, the lowest percentage of misassigned individuals corresponds to regions from the opposite coast. This indicates that few individuals venture from one coast to the other. For treatments E and F (Table 4-12), the results of the assignment test indicate that 50% and 63% of the individuals respectively were correctly assigned to their particular wintering area. In general, for treatments E and F, the highest percentage corresponded to correctly assigned individuals for all of the sites. For the Northwest, Central-West and the Southwest MPSWG sites, the proportion of the distribution of samples in relation to the sites to which they were designated was similar. These results strongly suggest intermixing of individuals among the wintering sites and genetic homogeneity within the gulf coast population, which in turn indicates that the three regions can be considered as one population. In comparing the Atlantic and the gulf coast (Northwest, Central-West, and Southwest) wintering sites, it was evident that strong frequency shifts in the distribution of

Table 4-10. Multilocus assignment test applied to individuals combined into two geographic areas, east coast (E) and west coast (W) of Florida. Treatments A and B.

	(*N*=223)*			(*N*=136)**		
Sampling area	E	W	*N*	E	W	*N*
East coast (E)	85 65%	46 35%	**131**	62 77%	19 23%	**81**
West coast (W)	12 13%	80 87%	**92**	11 20%	44 80%	**55**

* Analysis was performed taking into consideration all the individuals from east and west coasts with two or more scorable loci

** Analysis was performed taking into consideration individuals from the east and the west coasts with 7 or 8 scorable loci

N = number of individuals

Table 4-11. Multilocus assignment test for individuals in the five sampling areas, northeast (MNE), eastcentral (MEC), southeast (MSE), southwest (MSW) and northwest (MNW). Treatments C and D.

Sampling area	MNE	MEC	MSE	MSW	MNW	*N*=223
MNE	12 30%	8 20%	8 20%	6 15%	6 15%	**40**
MEC	6 14%	16 37%	1 2%	10 23%	10 23%	**43**
MSE	16 33%	5 10%	13 27%	6 13%	8 17%	**48**
MSW	2 44%	5 11%	1 2%	22 48%	16 35%	**46**
MNW	0 0%	8 17%	3 7%	13 28%	22 48%	**46**

Sampling area	MNE	MEC	MSE	MSW	MNW	*N*=136
MNE	6 29%	4 19%	8 38%	1 5%	2 10%	**22**
MEC	3 13%	10 42%	1 4%	5 21%	5 21%	**24**
MSE	14 40%	4 11%	11 31%	1 3%	5 14%	**35**
MSW	0 0%	1 5%	0 0%	13 59%	8 36%	**22**
MNW	1 3%	8 24%	1 3%	8 24%	15 45%	**33**

Table 4-12. Multilocus assignment test for individuals in five regions: Atlantic, Upper St. Johns, Southwest, Central-West, Northwest and Southwest. Treatments E and F.

Sampling area	Atlantic	Upper St. Johns	Central-West	Northwest	Southwest	***N* = 223**
Atlantic	73	9	15	9	23	**129**
	56.0%	7.0%	12.0%	7.0%	18.0%	
Upper St. Johns	1	1	0	0	0	**2**
	50.0%	50.0%				
Central-West	5	0	15	12	5	**37**
	14.0%		40.0%	32.0%	14.0%	
Northwest	1	0	5	4	1	**11**
	9.0%		46.0%	36.0%	9.0%	
Southwest	2	1	10	11	20	**44**
	5.0%	2.0%	23.0%	25.0%	45.0%	

Sampling area	Atlantic	Upper St. Johns	Central-West	Northwest	Southwest	***N* = 136**
Atlantic	60	2	7	7	3	**79**
	76.0%	3.0%	9.0%	9.0%	3.0%	
Upper St. Johns	0	1	0	0	0	**1**
		100.0%				
Central-West	4	0	11	9	2	**26**
	15.0%		42.0%	35.0%	8.0%	
Northwest	0	0	3	2	3	**8**
			37.5%	25.0%	37.5%	
Southwest	0	0	6	4	12	**22**
			27.0%	23.0%	50.0%	

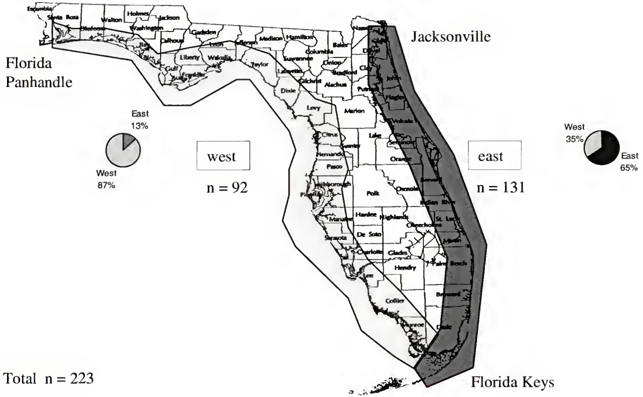


Figure 4-3. Map showing results on the multilocus assignment test performed on 223 samples collected along the east and the west coast of Florida.

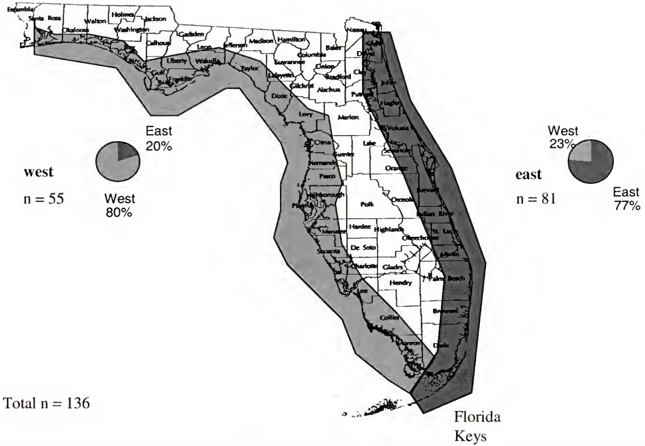


Figure 4-4. Map showing results on the multilocus assignment test performed on 136 samples collected along the east and the west coast of Florida.

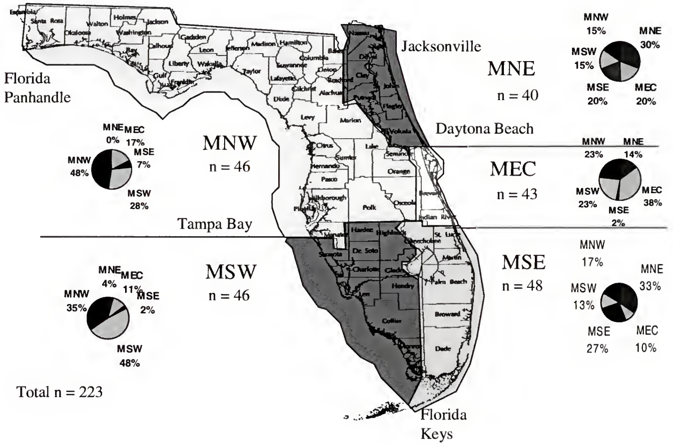


Figure 4-5. Map showing results on the multilocus assignment test performed on 223 samples distributed in the five sampling areas according to FFWCC.

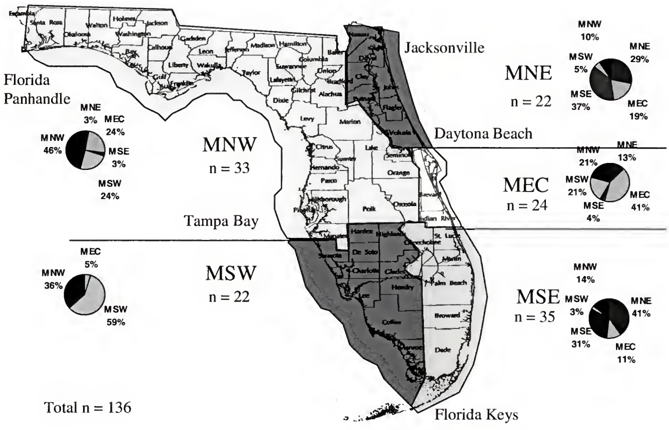


Figure 4-6. Map showing results on the multilocus assignment test performed on 136 samples distributed in the five sampling areas as described by the FFWCC.

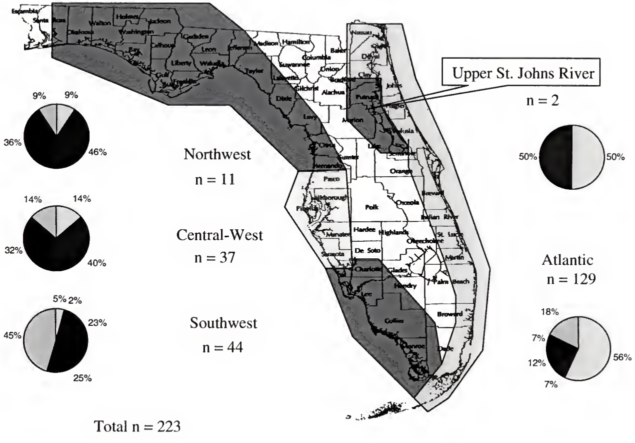


Figure 4-7. Winter aggregation regions proposed by the Manatee Population Status Working Group. Multilocus assignment test results based on microsatellite analysis.

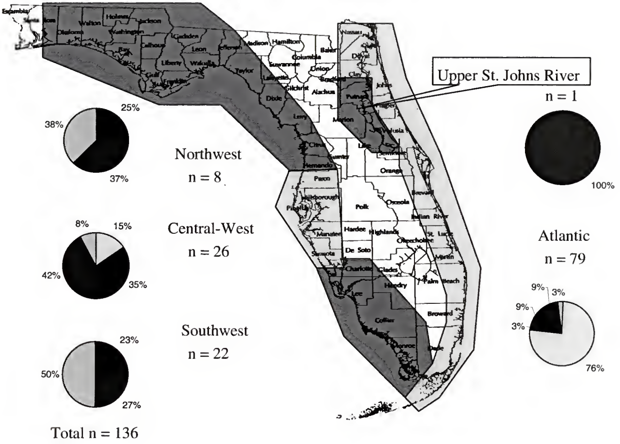


Figure 4-8. Geographic regions proposed by the Manatee Population Status Working Group. Multilocus assignment test results based on microsatellite analysis.

individuals are present, supporting the observation that although a significant geographic distance exists between these wintering sites, low levels of genetic intermixing occur between individuals from both coasts. It was not possible to make inferences for the Upper St. Johns River wintering site due to small sample size. It is recognized that this is a weakness in the study design, and that there is a need to further explore the genetic composition of the population of manatees that frequent this site. The Upper St. Johns River is used by manatees as a foraging ground throughout the year; manatees tend to aggregate in the Blue Spring area during the winter because it is a thermal refuge (O'Shea & Hartley, 1995). The ranges of the manatees studied from the Blue Spring and the Atlantic coast regions overlap, with some level of interchange occurring between the two populations (Reid et al.1991). An increment in the samples to be analyzed for the Upper St. Johns will provide further information on the amount of intermixing, which is crucial for the success of ongoing manatee management programs in these two important regions.

Population genetic structure and gene flow

Estimates by multilocus F_{st} were performed to explore levels of population structure between the east and west coasts (Table 4-13), and among the five regions (Tables 4-14 and 4-15). Pairwise F_{st} between the east and west coast were statistically different from zero, for both treatments A ($\chi^2 = 65.24$; d.f. = 7) and B ($\chi^2 = 66.83$; d.f. = 7) when analyzed with a chi-square goodness-of-fit test, supporting the hypothesis of population differentiation between the east and west coasts. Likewise, pairwise F_{st} values among the five regions, for both treatments C and D, provided similar results in that all of the pairwise comparisons between regions from opposite coasts (except for comparison MNW and MEC in treatment D) were statistically different from zero, indicating population differentiation (Tables 4-14 and 4-15). In addition, all of the pairwise comparisons performed between regions within the same coasts (except for MEC), for both treatments C and D, were not statistically significant (i.e., MNE and MSE, and MNW and MSW),

Table 4-13. Pairwise gene flow estimates in terms of *Nm* obtained from F_{st} estimates for treatments A (*N*=223) and B (*N* = 136).

EAST vs WEST N	F_{st} Estimate	χ^2	d.f.	Gene Flow *(Nm)*	Private allele method
223	0.0209	65.2	7	11.71	1.94
136	0.0351	66.3	7	6.87	0.3

A Chi-Square goodness-of-fit test was performed to evaluate if the F_{st} values are statistically different from zero.

$\chi^2 = 2NF_{st}(k-1)$ d.f. = (k-1)(s-1) ($P<0.05$)
where: *N* = total number of individuals
k = number of loci
s= number of populations

Table 4-14. Pairwise multilocus F_{st} estimates (below the diagonal). Chi-square goodness-of-fit test values and degrees of freedom (under F_{st} estimates). Gene flow estimates in terms of *Nm* obtained from F_{st} estimates are given in parenthesis (above the diagonal) for treatment C (*N* = 223).

	N	MNE	MEC	MSE	MSW	MNW
MNE	40		(8.97)	(24.50)	(18.16)	(5.67)
MEC	43	0.0271 *27.69* (d.f. = 7)		(10.00)	(10.00)	(15.18)
MSE	48	0.0101* *11.45 (d.f. = 7)*	0.024 *28.22 (d.f. = 7)*		(10.00)	(16.41)
MSW	46	0.0543 *60.05 (d.f. = 7)*	0.024 *27.55 (d.f. = 7)*	0.0242 *30 (d.f = 7)*		(48.70)
MNW	46	0.0422 *47.26 (d.f. = 7)*	0.0162 *18.82 (d.f. = 7)*	0.015 *19 (d.f = 7)*	0.0051* *6.25 (d.f. = 7)*	

* F_{st} values were not statistically different from zero. ($P<0.05$)
$\chi^2 = 2NF_{st}(k-1)$ d.f. = (k-1)(s-1) ($P<0.05$)
where: *N* = total number of individuals
k = number of loci
s = number of populations

Table 4-15. Pairwise multilocus F_{st} estimates (below the diagonal). Chi-square goodness-of-fit test values and degrees of freedom under F_{st} estimates. Gene flow estimates in terms of *Nm* obtained from F_{st} estimates are given in parenthesis (above the diagonal) for treatment D (N = 136).

	N	MNE	MEC	MSE	MSW	MNW
MNE	21		7.08		2.45	4.52
MEC	24	0.0341 *21.48 (d.f. = 7)*		8.71	6.73	22.07
MSE	35	-0.0003 0.00	0.0279 *23.04 (d.f. = 7)*		4.71	8.43
MSW	22	0.0923 *55.56 (d.f. = 7)*	0.0358 *23.05 (d.f. = 7)*	0.0504 *40.00 (d.f. = 7)*		14.03
MNW	34	0.0524 *40.34 (d.f. = 7)*	0.0112 *9.09 (d.f. = 7)*	0.0288 *27.82 (d.f. = 7)*	0.0175 *13.72 (d.f. = 7)*	

* F_{st} values were not statistically different from zero. (P<0.05)

$\chi^2 = 2NF_{st}(k-1)$ d.f. = (k-1)(s-1) (P<0.05)

where: N = total number of individuals
k = number of loci
s = number of populations

suggesting extensive gene flow and no population differentiation. Finally, the F_{st} estimate for the two most geographically distant populations (MNE and MNW) was 0.0422. In the multilocus-multisample test, the F_{st} value obtained was 0.0294.

Pairwise gene flow estimates obtained from the F_{st} values for treatments A and B were *Nm* = 11.71 and *Nm* = 6.87, respectively (Table 4-13). For treatments C and D migration levels were similar; within the east coast regions, migrations ranged from *Nm* = 24.5 between MNE and MSE, to *Nm* = 8.97 between MNE and MEC (Tables 4-14 and 4-15). The highest level of gene flow detected overall (*Nm* = 48.70) was between the two regions from the west coast. Levels of gene flow among east and west coast regions ranged from *Nm* = 16.41 between MNW and MSE, to *Nm* = 5.67 between MNE and MNW, the two most distant regions. For treatment D, the maximum level of migration observed was between regions MEC and MNW. Finally, levels of gene flow estimated from the multilocus multisample ($F_{st} = 0.0294$) yield a migration rate of $Nm = 8.25$.

Genetic distances between regions

Cavalli-Sforza and Edwards' chord distances (D_{ce}) obtained with the program NJBPOP were used to generate a genetic tree (Figure 4-9). Based on the results of genetic distance, regions from the west coast were clustered together 76% of the time based on 1,000 bootstrap replicates. Two regions from the east coast (MNE and MSE) clustered together in a different branch 45% of the time after 1,000 bootstrap replicates. The closest region to this cluster was the third population from the east coast (MEC).

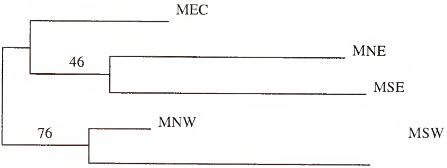
Cavalli-Sforza and Edwards' Chord distances (D_{ce}) obtained with the program BIOSYS generated a similar tree topology. Similar to the first tree, both populations from the east coast clustered together in one branch ($d = 0.119$). In addition, populations from the west coast clustered in a second branch ($d = 0.114$); however, population MEC was clustered with the west coast populations ($d = 0.035$, from node A to node B).

Inbreeding in the Florida manatee

Comparisons of observed vs. expected numbers of heterozygotes predicted at each locus under Hardy-Weinberg expectations indicate that heterozygote deficiencies occur in the east and west coast populations. In four out of eight (50%) locus-population comparisons, the observed number of heterozygotes were less than expected (Tables 4-6 and 4-7). Significant levels of heterozygosity deficiency was observed in the east coast population for both treatments A and B for loci TmaF14 ($\chi^2 = 12.99$, d.f. = 1), TmaE02 ($\chi^2 = 2.26$; d.f. = 3) and TmaA03 ($\chi^2 = 8.12$, d.f. = 1); and in treatment A for locus TmaM79 ($\chi^2 = 0.13$, d.f: 3). No heterozygoyte deficiency was observed for the west coast population in either treatment. Moreover, when pooling across all

Cavalli-Sforza and Edwards chord distance

1000 replicates



Cavalli-Sforza and Edwards chord distance

10000 replicates

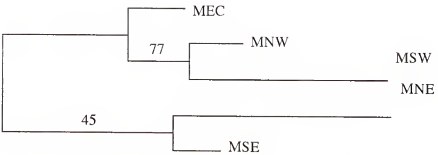


Figure 4-9. Cavalli-Sforza and Edwards' chord distance after 1,000 and 10,000 bootstrap replicates.

eight loci, no significant heterozygote deficit was found for the east or the west coast populations in either treatment A or B (Table 4-5). The inbreeding coefficient (F_{is}) for each locus was also calculated, which measures the extent of nonrandom mating within populations (Hart & Clark 1987). A few of the F_{is} values for both populations across all loci were greater than zero (Tables 4-6 and 4-7), indicating possible inbreeding within populations. Three out of eight values for the east coast in treatment A, and one in treatment B suggested non-random mating for the east coast population. Likewise, 37.5 % of the comparisons for the west coast were statistically greater than zero. However, when pooling across all loci, the F_{is} values were non-significant suggesting random mating within populations. In treatment C (Table 4-8), only TmaE08 for region MSW was out of HWE due to heterozygote deficiency ($\chi^2 = 22.45$, d.f. = 3). For treatment D (Table 4-9), locus TmaE08 showed an F_{is} value statistically different from zero, suggesting inbreeding for region MSW. Similarly, locus TmaF14 values of F_{is} were different from zero for region MEC and MSE. Finally, locus TmaA03 was different from zero for region MNE, suggesting non-random mating.

Discussion

Population Genetic Structure and Gene Flow in *Trichechus manatus latirostris*

This study on the microsatellite variation in the Florida manatee population provides two important findings. First, the geographically separated populations from the east and the west coasts of the peninsula are slightly different from each other in terms of their microsatellite genotypic and allelic frequency distribution. Second, high levels of gene flow and mixing occur among regions on both coasts. The results of this study contrast, at least to some degree, with earlier studies on manatee genetic analysis with allozymes. McClenaghan & O'Shea (1988) reported no population differentiation between the east and the west coasts, and no differentiation among the 5 regions studied. It is possible that the discrepancy between their results and the results in this study are due to the differences in sample sizes (59 vs. 223), in addition to the fact that microsatellites are more sensitive in detecting differentiation among closely related populations (Workman & Niswander 1970; Vigilant *et al.*1991). The results of mtDNA analyses showed one unique haplotype for the manatee population in Florida (n = 23). This suggested that a unique maternal lineage was established along both coasts of the peninsula as a result of a founder effect or a bottleneck event that most likely occurred in recent evolutionary time (Garcia-Rodriguez *et al.* 1998). In other words, not enough time has passed for genetic differentiation to occur in mtDNA. In addition to this, the results support radiotelemetry data suggesting that males and females move extensively along the coast of Florida, and thus the levels of migration and gene flow along the coasts are sufficient to keep both populations from diverging and differentiating into genetically distinctive units. Finally, the conclusions about the lack of population differentiation obtained with mtDNA analysis were based on a relatively small sample size ($n = 23$), which roughly represented about 1% of the minimum estimated number of manatees in Florida. It is very possible that additional haplotypes exist in low frequencies in this population but have yet to be detected. It will be informative to evaluate more individuals in order to determine the presence or absence of additional mtDNA haplotypes. It is also imperative to analyze the mtDNA haplotype of the samples used in this study so that a direct comparison of results using F_{st} as an estimator of differentiation can be used to explore the possibility of undetected low frequency haplotype variation. The amount of migration detected between the east and the west coast is enough to keep these two populations from diverging into two distinct evolutionary units. On the other hand, there appears to be a significant level of population differentiation, as expressed by F_{st} values and allele and genotypic frequencies. These results were consistently recovered in all treatments. Some level of migration is expected, as there have been documented isolated cases of wide-ranging coastal migration (Reid *et al.* 1991; Deutsch *et al.* 1998); however, it is unclear to what extent manatees venture between the Atlantic coast and the Gulf coast. Previous studies based on radiotelemetry suggest isolated events of intercoastal migration (Reid *et al.* 1995); however, the genetic implications of these movements were not known until now.

One explanation for the shallow, but significant level of genetic differentiation between the east and the west coast populations could be that each population is composed of a collection of closely related family groups. It is possible that several generations of these individuals within a family may stay together in the same coast, feeding and moving in a north/south fashion in response to water temperature changes. A second possibility is that individuals, especially females and their calves, establish fidelity to certain wintering areas. These sites are crucial for the survival of the species as they are used as warm water refuges during the winter. A high proportion of manatees have strong site-fidelity to these places, and there is also evidence that this behavior is passed from one generation to the next (Reid *et al.* 1995). This homing behavior might be an important component in shaping the loose family structure of manatees, and the tendency for closely related individuals to hang around in a common wintering area might have some influence on the level of differentiation observed between the east and west coasts. In addition to these, there is evidence of a break in the population distribution at the Florida Bay due to habitat unsuitability (Moore 1951). However, although rare, there is evidence that some individuals move from one coast to the other, and this movement may be enough to prevent deep population structure. A third explanation could be a combination of the scenarios described above. Analysis provided results that indirectly support the hypothesis of established populations on each coast. Pairwise and multilocus multisample F_{st} estimates, performed with different samples sizes (treatments C and D), support the suggestion that a shallow but statistically significant level of population differentiation exists between regions on the east (MNE, MSE) and on the west (MNW, MSW) coasts. The notable exception is that region MEC from the east coast cannot be differentiated from region MNW from the west coast. This is likely an artifact of the overall shallow levels of divergence.

What biological factors could be taking place that might have an effect on the differences in genotype and allele frequencies between the east and west coast populations but not among regions for each coast? Even though some manatees are known to undertake extensive movement, it is possible that a number of closely related individuals, especially sexually mature young adults, stay together within paternal or maternal areas because they have less experience (O'Shea & Hartley 1995). In addition, they have the resources and shelter they require so there is little need for extensive migration to other areas. Although it is too early to make definitive conclusions with respect to this scenario, the results certainly raise important questions that need attention in order to understand the dynamics that shape the fine-scale population structure within and between coasts.

Low Microsatellite Diversity as an Indicator of Recent Population History

One interesting finding of this study was the low number of alleles detected. Although additional loci should be screened and analyzed for the manatee, the genetic evidence presented in this study supports and extends the hypothesis that manatees probably did not occupy Florida during the most recent ice age. Garcia-Rodriguez *et al.* (1998) hypothesized that Florida may have been colonized after the last ice age by a small number of matrilines. The low diversity observed in microsatellite loci supports and extends this hypothesis and strongly suggests that a bottleneck effect occurred in recent evolutionary history.

Heterozygote Deficiency as a Measure of Non-random Mating in the Florida Population

Preliminary studies on heterozygote deficiency in manatees using allozymes (McClenaghan & O'Shea, 1988) indicated an excess of homozygosity between regions. These results indicate either inbreeding in populations or genetic subdivision between sample regions (Wahlund effect). The authors suggested an alternative explanation for the excess of homozygotes based on the fact that their samples originated from fatalities and may not be representative of the living population. The results in this study support their findings in that some level of heterozygote deficiency, expressed as significant F_{is} values, was observed in some regions. Historically, inbreeding has not been a major concern because manatees have a promiscuous breeding system (Hartman, 1979). A group of males will follow a female in estrous, and the female may successively copulate with more than one male (O'Shea and Hartley 1995). It is still too early to determine the level of relatedness among breeding individuals, but mating among closely related individuals may be taking place on a regular basis. Inbreeding may also be a historical factor if the contemporary Florida population was the result of a small number of closely related founders. The findings from mtDNA for the Florida manatee reported by Garcia-Rodriguez *et al.* (1998) support this argument. It is very likely that individuals that belong to the same family clan may have more opportunity for breeding, especially if both females and males stay together for long periods of times in the same region. This might explain the observed levels of heterozygosity deficiency, but more information on pedigree analysis is required to completely understand this pattern. A couple of alternative explanations can be proposed for these results: the presence of null alleles, which are alleles that cannot be amplified due to a mutation in the primer site, and nonrandom sampling of closely related individuals (Hansen *et al.* 1997). However, with data from this study it is not possible to evaluate the importance of these factors as causes of the observed heterozygote deficiencies. First, confirming the presence of null alleles is complicated, as it requires large-scale analyses of patterns of allele segregation in the populations. Secondly, it requires analysis of the sequence in the region of the genome where the null alleles are hypothesized to be present in both the homozygote and in the non-amplifying individuals, in order to see whether mutations, which prevent primer binding, have in fact occurred. To assess these alternative biological explanations for the observed heterozygote deficiencies, studies will be required that document reproductive success, as well as dispersal patterns and survival rates of juveniles and adults.

Conservation and Management Recommendations

The evidence provided in this study supports previous work by Garcia-Rodriguez *et al.* (1998) and McClenaghan & O'Shea (1988), in that the Florida manatee can be considered a single evolutionarily significant unit (ESU). It is further proposed that this ESU consists of two well-defined management units (Moritz 1994b), the east and the west coast manatee populations. Genetic evidence from this study showed that there was significant genetic differentiation between the samples when pooled into these two major geographically distinct populations. This was further supported by evidence of genetic differentiation when comparing regions from the east coast against regions from the west coast. The results of the multilocus assignment test indicate that 77% of the individuals sampled on the east coast were correctly assigned to that population. Similarly, 80% of the samples from the west coast were correctly assigned to that population (Table 4-10). Similar results were found in all treatments, suggesting that indeed these populations are genetically distinct although a low level of genetic exchange does occur between them. The results of this study support previous work by McClenaghan & O'Shea (1988) in that no genetic differentiation was detected among regions along either coast, suggesting extensive movement and gene flow within coasts. The assignment test constitutes additional evidence that supports extensive movement of individuals along each coast of the peninsula. These regions, based on winter aggregations, are useful for site-specific management purposes (McClenaghan & O'Shea 1988), but the microsatellite data indicates that they are not genetically distinct populations. Finally, it is important to explore the Florida manatee genetic structure at a finer scale; that it is at the family level, to elucidate social structure and reproductive behavior. Determining the frequency of the different lineages or family units present in each geographic area, as well as the level of genetic flow among them, will have far-reaching conservation implications. Some management decisions may benefit from the recognition of family relationships within geographic or wintering groups. For example, should depleted manatee populations be supplemented by individuals translocated from other areas? Should a rehabilitated manatee be released indiscriminately in any appropriate region?. This maintenance of genetic heterogeneity will increase overall population fitness, reduce disease susceptibility, and have a major impact on the long-term survival for this endangered species.

CHAPTER 5
SUMMARY AND CONCLUSIONS

Three studies were undertaken to determine the genetic makeup of the West Indian manatee (*Trichechus manatus*). The first study determined the evolutionary relationships among geographically distinct populations distributed along the Caribbean and North and South America. This was accomplished by employing mitochondrial DNA sequences as a genetic marker to trace the evolutionary history of the species. In the second study, microsatellite genetic markers specific for manatees were developed for studies on population structure and to determine the level of recent gene flow between putative populations of the Florida manatee (*T. m. latirostris*). The third study was designed to explore further the levels of genetic relatedness between manatee populations along the east and west coasts of Florida and to evaluate the levels of genetic differentiation among discrete regions of manatee concentrations, which have historically been used by researchers for conservation and management purposes. The major conclusions for each study and recommendations for future studies are presented below.

Phylogeography of the West Indian Manatee (*Trichechus manatus*): How Many Populations and How Many Taxa?

Population genetic structure and phylogeography of the West Indian manatee (*T. manatus*) was elucidated using mtDNA control region sequences. Comparison among eight regions across the western Atlantic region identified fifteen haplotypes among 86 individuals from Florida, Puerto Rico, the Dominican Republic, Mexico, Colombia, Venezuela, Guyana and Brazil. Strong population separations between most regions were demonstrated with significant haplotype frequency shifts. These findings suggest that stretches of open water and unsuitable coastal habitats constitute substantial barriers to gene flow and colonization, in spite of the potential for long distance mobility as demonstrated by radiotelemetry studies. Low levels of genetic diversity within the Florida and Brazilian samples are explained by either recent colonization into high latitudes or bottleneck effects. Three distinctive mtDNA lineages were observed in an intraspecific phylogeny of *T. manatus*, corresponding approximately to 1) Florida (U.S.A) and the West Indies, 2) the Gulf of Mexico to the Caribbean rivers of South America, and 3) the northeast Atlantic coast of South America. These lineages, which are not concordant with previous subspecies designations, are separated by sequence divergence estimates of d=0.04-0.07, which is approximately the same level of divergence observed between *T. manatus* and the Amazonian manatee (*T. inunguis*, $n = 16$). Three individuals from Guyana, identified as *T. manatus*, had mtDNA haplotypes that are affiliated with the endemic Amazon form of *T. inunguis*. The three primary *T. manatus* lineages and the *T. inunguis* lineage may represent relatively deep phylogeographic partitions, which have been bridged recently due to changes in habitat availability (after the Wisconsin glacial period, 12 thousand years BP), natural colonization, and human-mediated transplantation. The results from surveyed manatee populations confirm the demographic independence of isolated populations and provide an approximate yardstick to define management units throughout the range of *T. manatus*. Furthermore, the mtDNA data presented here provide a strong motivation for further investigation to explore whether the separations based on mtDNA phylogeny are supported by other genetic and ecological lines of evidence. This is important as conservation priorities may be adjusted to include three or maybe more evolutionary units for *T. manatus*.

Isolation and Characterization of Microsatellite DNA Markers in the Florida Manatee (*Trichechus manatus latirostris*) and their Application in Selected Sirenian Species

Microsatellite markers were developed specifically for the Florida manatee (*T. m. latirostris*) to explore finer-scale population subdivisions that might exist within populations of manatees distributed along the east and the west coasts of the Florida peninsula.

Fifteen sets of microsatellite primers amplified consistently scorable fragments of expected size with Florida manatee genomic DNA. These markers were screened using 50 manatee samples collected throughout the Florida peninsula. Eight of the 15 loci were polymorphic in this survey and overall levels of heterozygosity averaged 41.2%. Low levels of allelic diversity were observed in the Florida manatee. The maximum number of alleles identified at the most polymorphic locus (TmaE11) was six, and the average number of alleles observed in polymorphic loci was 2.9. This paucity of genetic diversity is indicative of a founder effect or major population bottleneck of evolutionary significance and is consistent with findings from the mtDNA study. In comparison to published reports on related terrestrial and marine mammals, the Florida manatee exhibited considerably less allelic diversity. Indeed, this study reports one of the lowest levels of genetic diversity observed with microsatellite markers. Regardless of the low level of genetic diversity, the markers provide enhanced resolution of the population structure.

Cross-species amplification was tested in three Sirenian species: the Antillean manatee (*T. m. manatus*), the Amazonian manatee (*T. inunguis*), and the dugong (*Dugong dugong*). Eleven of 15 markers were polymorphic for the Antillean and the Amazonian manatee. Although at least nine markers were polymorphic in the dugong, estimates of polymorphism are likely to be underestimated due to the small sample size. This suite of markers appears to be ideal for identification of population structure and possibly pedigree analysis in all four Sirenian species, thus providing a nuclear DNA-based approach to complement existing mtDNA genetic information for these vulnerable species.

Population Genetic Structure and Gene Flow in *Trichechus manatus latirostris*

The study on the microsatellite variation in the Florida manatee population provides two important results. First, the geographically separated (but incompletely isolated) populations from the east and the west coasts of the peninsula are slightly different from each other in terms of their microsatellite genotypic and allelic frequency distributions. Second, high levels of gene flow and mixing occur among individuals within both east and west coast populations. The results of this study corroborate, at least to some degree, earlier studies on manatee genetic analysis using allozymes and mtDNA. It is very possible that the discrepancies between these two studies and the results presented here might be due to differences in sample sizes, and that insufficient time has passed for genetic differentiation at allozyme loci and mtDNA. In addition, microsatellites are more sensitive at detecting differentiation among closely related populations.

The amount of migration detected between the east and west coast is enough to keep these two populations from diverging into distinct evolutionary units. On the other hand, there appears to be a significant level of population differentiation, as expressed by F_{st} values and allele and genotypic frequencies. Some level of migration is expected, as there have been a few documented cases of wide-ranging coastal movement (Reid *et al.* 1991; Deutsch *et al.* 1998); however, it is unclear to what extent manatees venture between the Atlantic coast and the Gulf coast. Previous studies based on radiotelemetry suggest isolated events of intercoastal migration (Reid *et al.* 1991; Deutsch *et al.* 1998), however, the genetic implications of these movements were not known until recent.

One explanation for the shallow, but significant level of genetic differentiation between the east and the west coast regions could be that each population is composed of a collection of closely related family groups. A second explanation is that individuals, especially females and their calves, established fidelity to certain wintering areas. These wintering sites are crucial for the survival of the species during the colder months of the year (November to late March). This homing behavior might be an important component in shaping the loose family structure of manatees, and the tendency for closely related individuals to occupy a common wintering area might have some influence on the level of differentiation observed between the east and west coasts of Florida. A third explanation might be found in the fact that a distribution break exists at the Florida Bay. However, there is also evidence that some individuals move from one coast to the other, and this movement may be enough to keep these populations from experiencing complete genetic isolation. Analysis of the level of population differentiation between regions, both within and between each coast, also provides encouraging results that indirectly support the hypothesis of established east and west coast populations for the Florida manatee.

One interesting finding of this study was the low level of alleles detected for all the loci. Although additional loci should be screened and analyzed for the manatee, the trend in the findings point to a possible bottleneck or founder effect in the Florida population, followed by some level of breeding among closely related individuals. However, it is too soon to make definitive conclusions and additional studies will be necessary in order to address this possibility.

Preliminary studies on heterozygote deficiency in manatees using allozymes indicated an excess of homozygosity between regions. These results indicate either inbreeding in populations or genetic subdivision between samples (Wahlund effect). The results of this study support the finding that some level of heterozygote deficiency is present in the population. Inbreeding should be considered as an explanation, in spite of the fact that manatees have a promiscuous breeding system, and based on the assumption that the contemporary Florida manatee population was the result of successful breeding among a small number of founders. The findings from mtDNA for the Florida manatee support this argument. The relatedness of individuals might explain the observed levels of heterozygosity deficiency, but more information on pedigree analysis is required to fully understand this pattern. Several alternative explanations can be proposed for these results: 1) the presence of null alleles, which are alleles that cannot be amplified due to a mutation in the primer site, and 2) nonrandom sampling of closely related individuals. However, with the data from this study it is not possible to evaluate the importance of these factors as causes of the observed heterozygote deficiencies.

Conservation and Final Recommendations for Future Studies

Effective conservation and restoration plans require clearly definable units of management. Currently, Florida manatee management units are defined as regions along the Atlantic and Gulf coasts of Florida and the upper St. Johns River. However, isolation is usually not complete and since most organisms have some power of dispersal, members of a local population have a low but positive probability of interaction with individuals from other regions. Depending on the rate of migration, demographic and genetic dynamics will be influenced by this migration, as well as by local birth and death rates. A low intrinsic reproductive rate and low natural population density make the Florida manatee particularly vulnerable to human perturbations, as ongoing habitat loss and high mortality rates continue to threaten the future of the species. One consequence of population reduction is loss of genetic diversity since several generations of severe inbreeding in a small population, or repeated crashes to a few individuals, can deplete most of the genetic variation from an initially larger population. These studies provide preliminary results that suggest that inbreeding has occurred in the Florida manatee population; however, it is important to explore these findings further by screening additional microsatellite markers, which will allow for a finer genetic differentiation between individuals. This information is crucial for pedigree analyses which will provide detailed information on the levels of genetic relatedness of breeding individuals. Genetic studies of family units, pedigrees, social behaviors, and effective population size are required to better understand the dynamics of gene flow among regions. This is essential for maintaining evolutionarily significant manatee lineages to ensure long-term population stability and reduce the need for further protection through regulatory processes.

Human activities are related to at least one third of all manatee mortalities (collisions with boats, entanglement in fishing gear, and drowning or crushing in navigation locks or flood-control gates). Extensive information on the physiology and pathology of the manatee has been gathered from carcasses resulting from those events, as well as from natural causes. At present, no genetic foundation for forensic investigation exists. Once the subpopulations are surveyed, this information will allow identification of carcasses in the context of family unit and geographic origin.

Individual Florida manatees periodically undertake long distance migrations of up to 2000 km, (as far west as Texas and as far north as Rhode Island). Some of these individuals have been rescued, radio-tagged and released in Florida so that their movements can be monitored. Recently, with the use of mitochondrial DNA analysis, it was possible to determine the most probable population origin for a manatee that was found and rescued from Texas waters. This manatee was identified as a member of the Florida stock and actions were taken to repatriate her. The genetic information available at that time made it possible to determine the population of origin; however, it was not possible to identify the most probable family or geographic origin within Florida. More extensive microsatellite data (with perhaps 8 additional polymorphic loci) would allow repatriation to a specific feeding area or winter refuge.

A number of manatees have been born in captivity or held in captivity for many years (e.g., those rescued as orphaned calves). These manatees will ultimately be released in the wild. The information gathered from microsatellite markers will allow researchers to develop better release criteria and to predict potential post-release success.

Finally, Florida manatee population structure and reproductive behavior can be defined by determining the frequency of the different lineages or family units present in each geographic area, as well as by determining the level of genetic flow among those areas, and this knowledge will have far-reaching conservation applications. Some management decisions may benefit from the recognition of family relationships within geographic or wintering groups. For example, should depleted manatee populations be supplemented by transplantation from other areas? Should a recovered manatee be released indiscriminately in any appropriate region? Misdirected transplantation or translocation could compromise the integrity of genetic differences observed between the east and west coast populations. This maintenance of genetic heterogeneity will increase overall population fitness, reduce disease susceptibility, and have a major impact on the long-term survival prospects for this endangered species.

APPENDIX

LIST OF MANATEE CARCASS SAMPLES USED FOR THIS STUDY

Date	Field ID	FFWCC region	Assigned region	MPSWG region	Assigned region	Sex	Length	State	County	Waterway	Latitude degrees	Longitude degrees	Locality
970606	MEC9732		■			M	136.8	FL	Brevard	Banana River	28	80	Merritt Island, located directly behind residence at 1355 Fiddler Ave.
970815	MEC9750				■	F	378	FL	Seminole	Lake Monroe	28	81	Sanford, on the south shoreline of the Lake Monroe between the Hospital and I-4.
970916	MEC9754		▒			M	135	FL	Volusia	Halifax River	29	81	Daytona Beach, south of Main Street Bridge, under wooden-walkway bridge.
970929	MEC9759		░		■	M	102	FL	Brevard	Turkey Creek	28	80	Palm Bay, in canal behind residence at 897 Vance Cr.
971201	MEC9767		■		■	M	168	FL	Brevard	Indian River	28	80	Port St. John, FPL Power Plant intake Canal fence.
971201	MEC9768		■		■	M	288	FL	Brevard	Banana River	28	80	Canaveral, NE corner of SR528, just south of lock gate at Port Canaveral.
971201	MEC9769					M	176	FL	Brevard	Banana River	28	80	Merritt Island, in canal behind 1685 Sandpiper Street.
971201	MEC9770		▒		■	M	272	FL	Brevard	Banana River	28	80	Merritt Island, behind 1415 Fiddler Avenue.
971224	MEC9776					M	199	FL	Indian River	Sebastian River	27	80	Sebastian, south side of the Sebastian Inlet.
980113	MEC9777				▓	F	289	FL	Volusia	Atlantic Ocean	29	81	Ormond-By-The-Sea, beached 150 yards north of North Lifeguard Station. North of Daytona Beach
980228	MEC9806		▒		■	M	256	FL	Brevard	Banana River	28	80	Kennedy Space Center, approximately 1 1/2 miles south of security boat dock at Mobile Support Structure.
980228	MEC9808					M	133	FL	Brevard	Indian River	28	80	Melbourne, directly behind residence at 6216 Halyard Ct.

Date	Field ID	FFWCC region	Assigned region	MPSWG region	Assigned region	Sex	Length	State	County	Waterway	Latitude degrees	Longitude degrees	Locality
980228	MEC9809		█			M	179	FL	Brevard	Banana River	28	80	South Patrick, located in first canal south of Pineda Causeway on eastern shore of Banana River.
980408	MEC9810				▒	F	132	FL	Indian River	Indian River	27	80	Vero Beach, carcass was located in first canal off of Bethel Creek in the Indian River.
980415	MEC9812		█			M	119	FL	Brevard	Banana River	28	80	Merritt Island, directly across from 630 Milford Point Drive, in Grand Canal, north of SR 520.
980415	MEC9813		░			F	213	FL	Brevard	Indian River	28	80	Port St. John, FPL Power Plant at intake well 2A (east well).
980424	MEC9814		▒			M	253	FL	Brevard	Banana River	28	80	Merritt Island, Kars Park (MINWR), approximately 1/2 mile south of boat ramp.
980424	MEC9815		▒		█	F	223	FL	Indian River	Indian River	27	80	Sebastian, in the Indian River approximately 1 1/2 mile north of Wabasso Road on the west shoreline.
980519	MEC9816					M	101	FL	Brevard	Sykes Creek	28	80	Merritt Island, east of Sykes Creek Bridge, behind residence at 545 Barrett Drive.
980519	MEC9818				▓	M	139	FL	Brevard	Banana River	28	80	Merritt Island, directly behind residence at 1555 Surfside Blvd, in a canal off the Banana River.
980519	MEC9819				▓	M	87	FL	Brevard	Banana River	28	80	Merritt Island, directly behind residence at 1385 Angler Street in canal off the Banana River.
980519	MEC9820		░			F	140	FL	Brevard	Indian River	28	80	Pineda, west entrance of Sun Harbor Marina at 6155 N. US1 in Indian River.
980430	MEC9821					M	105	FL	Brevard	Eau Gallie River	28	80	Melbourne, behind Spring Creek Condos off 100 Eau Gallie Blvd., west of US1 railroad tracks.

Date	Field ID	FFWCC region	Assigned region	MPSWG region	Assigned region	Sex	Length	State	County	Waterway	Latitude degrees	Longitude degrees	Locality
980618	MEC9822		▓		▓	M	111	FL	Brevard	Sykes Creek	28	80	Merritt Island, in canal off Sykes Creek directly behind residence at 254 Sykes Pt. Lane.
980629	MEC9823		▓			M	308	FL	Volusia	Halifax River	28	80	Oak Hill, directly behind Bissit Fish Camp, 251 Bissit Bay.
980629	MEC9824		▓			M	321	FL	Brevard	Banana River	28	80	Merritt Island, in the Banana River, southeast of SR528, approximately 200 yards on shoal.
980527	MEC9828		▒			F	146	FL	Brevard	Sykes Creek	28	80	Merritt Island, located in a canal behind 207 Sykes Point Lane off of Sykes Creek.
980605	MEC9829				▓	F	148	FL	Brevard	Banana River	28	80	Cocoa Beach, located in a canal off the Banana River, at the end of South 8th Street.
980629	MEC9830		■			M	215	FL	Brevard	Grand Canal	28	80	Satellite Beach, in canal off the Grand Canal directly behind residence at 755 Loggerhead Island Way in the Banana River.
980710	MEC9832					F	187	FL	Brevard	Banana River	28	80	Canaveral Air Force Station, off Air Force hanger catwalk.
980710	MEC9833		■			M	212	FL	Volusia	ICW	29	80	New Smyrna Beach, ICW, in the mouth of the second channel north of the north bridge.
980710	MEC9834		■			F	254	FL	Brevard	Sykes Creek	28	80	Merritt Island, Hampton Homes Subdivision, behind 330 Riverside Avenue canal.
980613	MEC9836		▓		■	F	123	FL	Brevard	Grand Canal	28	80	Satellite Beach, in the canal between Cinnamon Ct. and Kenwood Ct., directly behind residence at 625 Kenwood Ct.
980711	MEC9837		■			M	198	FL	Brevard	Banana River	28	80	Cocoa Beach, in canal off the Banana River, directly behind residence at 748 Nassau Road.

Date	Field ID	FFWCC region	Assigned region	MPSWG region	Assigned region	Sex	Length	State	County	Waterway	Latitude degrees	Longitude degrees	Locality
980711	MEC9838		░			M	195	FL	Brevard	Indian River	28	80	Melbourne, approximately 1/4 mile south of Intracoastal Marine, west bank, approximately 200 yards off shore.
980711	MEC9839		█		▓	F	133	FL	Brevard	Banana River	28	80	Cocoa Beach, in a canal between Banana River Blvd. and St. Croix Street.
980805	MEC9841				▒	M	276.5	FL	Volusia	Halifax River	29	80	New Smyrna Beach, ICW, south of US Coast Guard Station, next to marker #19.
980807	MEC9842				▓	M	292	FL	Volusia	Halifax River	29	80	Port Orange, against sea wall at Riverside Drive and Fleming Avenue intersection.
980902	MEC9843		▒		▒	F	352	FL	Brevard	Indian River	28	80	Eau Gallie, south of causeway approximately 100 yards south of boat camp on east side of ICW.
980901	MEC9844				▓	M	285	FL	Brevard	Port Canaveral Inlet	28	80	Port Canaveral, east of Canaveral Locks, southside of Port.
980904	MEC9845		░			M	296	FL	Brevard	Port Canaveral	28	80	Port Canaveral, under docks behind cruise terminal #4 (Canaveral Cruise Line).
981013	MEC9846		▓		▓	F	222	FL	Indian River	Indian River	27	80	Vero Beach, located in Indian River approximately 300 yards from McWilliams Park.
980921	MEC9849					F	353	FL	Volusia	St. Johns River	28	81	Orange City, Blue Springs State Park, approximately 300 yards south of the mouth of the spring in the lagoon area.
920721	MNE9216	░	▓			F	235	FL	Clay	Doctors Lake (St. Johns River)	30	81	Orange Park, N. shore of Doctors Lake, W. of Doctors Inlet, E. of Wadesboro Springs, N. of Romeo Point.

Date	Field ID	FFWCC region	Assigned region	MPSWG region	Assigned region	Sex	Length	State	County	Waterway	Latitude degrees	Longitude degrees	Locality
920730	MNE9218	▨	■			M	227	FL	Clay	St. Johns River	30	81	Orange Park, Villas Continental and Yacht Club, 2223 Astor St., Orange Park, FL 32073. At the mouth of Johnson Slough.
960116	MNE9602	▨				F	312	FL	Duval	St. Johns River	30	81	Jacksonville, on north bank between Dames Point Bridge and Dunns Creek.
961120	MNE9618	▨			■	M	353	FL	Duval	Ortega River	30	81	Jacksonville, on the rocks on the southeast side of the Hwy. 17 Roosevelt Bridge.
961126	MNE9619	▨			■	M	278	FL	Duval	St. Johns River	30	81	Jacksonville, adjacent to the old University Country Club, at the North 400 block of University Boulevard.
970422	MNE9704	▨				M	138	FL	Clay	Doctors Lake	30	81	Orange Park, behind residence at 2481 Sylvan Chanse Road.
970607	MNE9706	▨				M	104	FL	Flagler	Palm Coast Canals	29	81	Palm Coast Canals, slip #34 in the northeast portion of the canal, the east-central portion of the canal system.
970610	MNE9707	▨				M	301	FL	St. Johns	Robinson's Creek	29	81	St. Augustine, at the northern end of the creek in a tributary off of the west end of the Tolomato River (ICW).
970616	MNE9708	▨	■			M	102.5	FL	Duval	Pottsburg Creek	30	81	Jacksonville, behind the residence at 2045 Mills Ct. in a canal off of the creek, east of the St. Johns River.
970713	MNE9710	▨	▨			M	160	FL	Duval	San Pablo River ICW	30	81	Jacksonville, in a marshy area on the west bank of the ICW behind the residence at 14750 Beach Blvd.
970717	MNE9711	▨	■		▨	M	286	GA	Camden	Atlantic Ocean	30	81	St. Mary's, in the ocean offshore.

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970804	MNE9713	▨	░	░	▩	M	305	FL	Duval	St. Johns River	30	81	Jacksonville, at the mouth of the Trout River under the I-17 Bridge behind Jackie's Seafood.
970807	MNE9714	▨	▨	░	▨	F	348	FL	Duval	St. Johns River	30	81	Jacksonville, in the St. Johns River approximately 100 yards south of Point La Vista.
970815	MNE9715	▨	▨	░	░	F	133	FL	Flagler	Flagler Beach-ICW	29	81	Flagler Beach, in a residential canal behind 104 Palm Circle.
970929	MNE9716	▨	▨	▨	░	M	307	FL	Putnam	St. Johns River	29	81	Palatka, Browns Landing, marker "23" on west bank along shoreline.
971017	MNE9717	▨	░	░	░	M	298	FL	Duval	Pablo Creek	30	81	Jacksonville, 300 yards south of J.T. Butler Blvd. on the west bank of the ICW.
980203	MNE9802	▨	▩	░	▩	F	217	FL	Duval	St. Johns River	30	81	Jacksonville, behind business at 111 Riverside Ave.
980317	MNE9813	▨	▨	░	░	F	315	FL	Nassau	Amelia River	30	81	Fernandina Beach, behind 231 North Front Street on the east bank of Amelia River
980401	MNE9817	▨	■	░	■	M	126	FL	Duval	St. Johns River	30	81	Jacksonville, south of Goodby's Creek, behind 3745 Beauclerc Circle North.
980429	MNE9818	▨	■	░	▨	M	303	FL	Clay	St. Johns River	29	81	Green Cove Springs, floating near Pier 11 at the Green Cove Springs Marina, 851 Bulkhead Rd.
980702	MNE9821	▨	▩	░	■	U	65	FL	Duval	Trout River	30	81	Jacksonville, behind 8132 Trout River Drive.
980711	MNE9824	▨	▩	░	■	M	228	FL	Flagler	ICW	29	81	Palm Coast, behind Hammock Hardware, 2 miles north of the Hammock Dunes Bridge on the east side of the ICW

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980727	MNE9827		■			F	303	FL	Volusia	Halifax River-ICW	29	81	South Daytona Beach, behind residence at 139 Tradewinds Court.
980727	MNE9828		▨		■	M	272	FL	Volusia	Halifax River-ICW	29	80	South Daytona Beach, 100 feet east of ICW marker #48.
980725	MNE9829				▨	M	134	FL	Clay	North Fork Black Creek	30	81	Middleburg, North Fork Black Creek, behind 4144 Scenic Drive.
090898	MNE9831					M	111	FL	Flagler	ICW	29	81	Beverly Beach, approximately 3 miles north of the Moody Boat Ramp in the Sea Ray Canal on the west side of the ICW.
980929	MNE9835					F	292	FL	Duval	Ortega River	30	81	Jacksonville, behind a residence at 5036 Sanibel Drive.
981021	MNE9836				■	M	181	FL	St. Johns	St. Johns River	30	81	Switzerland, St. Johns River, at the end of St. Johns River Road floating in the grass along the eastern bank of the river.
	MNE9842		■		■								
990115	MNE9901					F	194	FL	St. Johns	Atlantic Ocean	29	81	St. Augustine, Butler Beach, between St. Augustine and Matanzas Inlets, south of Mary Street.
990113	MNE9902		▨		■	F	146	FL	Clay	St. Johns River	30	81	Orange Park, behind 5973 Pine Avenue, along bulkhead.
990201	MNE9903		▨		▨	M	176.5	FL	Clay	Doctors Lake	30	81	Orange Park, behind residence at 2518 Plainfield Road.
990126	MNE9904		▨			M	245	FL	St. Johns	St. Johns River	29	81	Elkton, behind residence at 2055 County Road 13 South.
990204	MNE9905		■			F	254	FL	St. Johns	St. Johns River	29	81	Picolata, south of Hwy 16 on east side of St. Johns River, 0.8 miles south of Colee Cove Road, along the river bank.

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990224	MNE9907					F	385	FL	Putnam	Cross Florida Barge Canal	29	81	Satsuma, floating 0.8 miles ENE of the Buckman Lock, off of the St. Johns River.
990605	MNE9910					M	318	FL	St. Johns	Palm Valley-ICW	30	81	Palm Valley, 200 yards south of Palm Valley Bridge (HWY210), on west shoreline of ICW.
990605	MNE9914					M	230	FL	Duval	St. Johns River	30	81	Jacksonville, at the east end of the Jacksonville Port Authority coal dock.
990510	MNE9915					M	122.5	FL	Flagler	Palm Coast Canals	29	81	Palm Coast, floating behind residence at 3 Cherrytree Ct.
990516	MNE9916					F	297	FL	St. Johns	Tolomato River	30	81	Palm Valley, 1.5 miles south of Palm Valley Bridge (SR210), east side between marker 9 and 10.
990702	MNE9917					M	286	FL	St. Johns	San Sebastian River	29	81	St. Augustine, west bank of San Sebastian River across from St. Augustine Marine at 404 Riberia St.
940315	MNW9407					M	104	FL	Pasco	Anclote River	28	82	Holiday, in a residential canal behind 5514 Riddle Road.
961212	MNW9626					F	286	FL	Hillsborough	Hillsborough Bay	27	82	Apollo Beach, beached at the high water line on the south bank at the entrance to the TECO power plant discharge canal. S of Tampa
970116	MNW9701					F	320	FL	Hillsborough	Hillsborough Bay	27	82	Tampa, Port Sutton channel, approximately half way down the channel on the north side.
970128	MNW9703					F	300	FL	Hillsborough	Alafia River	27	82	Gibsonton, under the pier at Williams Park at the intersection of US41 and the Alafia River. South of Tampa
970207	MNW9705					F	356	FL	Hillsborough	Alafia River	27	82	Gibsonton, between the dock and the sea wall behind residence at 9930 Altavista Dr. S of Tampa

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970217	MNW9706					F	127	FL	Pinellas	Mullet Key Bayou	27	82	St. Petersburg, Ft. DeSoto, at the bottom of a shallow lagoon. S of Tampa
970218	MNW9707					M	341	FL	Pasco	Anclote River	28	82	Anclote, against the debris barrier of the intake canal at the Anclote Power Plant.
970319	MNW9709					M	119	FL	Citrus	Dixie Bay	28	82	Dixie Shores, behind residence at 11739 Bayshore Drive.
970324	MNW9710					F	265	FL	Hillsborough	Little Manatee River	27	82	Ruskin, floating against the north bank of the river at the elbow off 7 Street SW. S of Tampa
970512	MNW9711					M	138	FL	Taylor	Steinhatchee River	29	83	Steinhatchee, behind residence at 467 Granger Drive on northwest side of bridge across the Steinhatchee River.
970520	MNW9712					F	105	FL	Pinellas	Clearwater Harbor	27	82	Clearwater, floating at the end of a dock at the Clearwater Marina on Seminole Street.
970603	MNW9714					M	153	FL	Citrus	Indian River	28	82	Crystal River, floating in the river between Timucua Pt. N and Apalachee Pt. N.
970818	MNW9717					F	263	FL	Hillsborough	Rocky Creek	27	82	Tampa, floating behind the residence at 9022 W. Hillsborough Avenue.
971021	MNW9718					M	330	FL	Pinellas	Tampa Bay	27	82	St. Petersburg, Coquina Key, N. end of Coquina Key Arms South apartment complex, in canal between the complex & residential area. S of Tampa
971113	MNW9719					F	277	FL	Hillsborough	Tampa Bay	27	82	Apollo Beach, approximately due east of green channel marker #11, along the shoreline. S of Tampa
971201	MNW9720					F	201	FL	Manatee	Sarasota Bay	27	82	Longboat Key, behind residence at 5030 Gulf of Mexico Drive. S of Tampa

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971201	MNW9721					M	215	FL	Hillsborough	Tampa Bay	27	82	Apollo Beach, on park property at the north end of Surfside Blvd., directly across from the TECO power plant. S of Tampa
971224	MNW9724					F	258	FL	Citrus	Kings Bay	28	82	Crystal River, east side of Buzzard Island, opposite the mouth of Crystal River.
980102	MNW9725					M	135	FL	Manatee	Tampa Bay	27	82	Terra Ceia, 50 yards southeast of the South Skyway Bridge Rest Area. S of Tampa
980203	MNW9801					M	322	FL	Hillsborough	Alafia River	27	82	Gibsonton, near spoil island north of the mouth to the Alafia River in 1 foot of water. S of Tampa
980210	MNW9802					M	305	FL	Hillsborough	Hillsborough Bay	27	82	Apollo Beach, ELAPP reclaimed area at north end of Surfside Blvd. on beach, entrance to TECO power plant discharge canal. S of Tampa
980415	MNW9804					F	132	FL	Pinellas	Whitcomb Bayou	28	82	Tarpon Springs, in the mangroves across from residence at 409 Whitcomb Blvd.
980416	MNW9805					M	288	FL	Pasco	Gulf of Mexico	28	82	New Port Richey, on a private beach at the end of Floramar Terrace South, due west of the circle drive at the beach.
980417	MNW9806					M	126	FL	Pasco	Anclote River	28	82	Holiday, in a canal off the Anclote River off Chancellor Drive.
980519	MNW9807					F	130	FL	Manatee	Braden River	27	82	Bradenton, Horseshoe Cove Trailer Park, on northeast corner of island across from River Run. S of Tampa

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980519	MNW9808					F	121	FL	Hillsborough	Tampa Bay	27	82	Ruskin, off a vacant lot at the corner of Dickman Drive and Campus Drive, off Ruskin Inlet. S of Tampa
980522	MNW9809					F	155	FL	Citrus	Homosassa River	28	82	Homosassa, River Haven Village, in a residential canal off the Homosassa River.
980529	MNW9810					F	332	FL	Hillsborough	Old Tampa Bay	27	82	Tampa, Rocky Point, floating behind the Double Tree Inn.
980702	MNW9811					F	127	FL	Hillsborough	Alafia River	27	82	Brandon, behind residence at 4006 Alafia Blvd. S of Tampa
980707	MNW9812					F	315	FL	Pinellas	Old Tampa Bay	27	82	St. Petersburg, floating under the mangroves on the west side of Big Island. S of Tampa
980707	MNW9813					F	247	FL	Hillsborough	Old Tampa Bay	27	82	Tampa, beached just west of the entrance to the channel directly across from the boat ramp on the Courtney Campbell Causeway.
980707	MNW9814					F	328	FL	Manatee	Perico Bayou	27	82	Palma Sola, inside the mouth of Perico Bayou by Mead Point. S of Tampa
980714	MNW9815					U	112	FL	Hernando	Weeki Wachee River	28	82	Weeki Wachee, in a residential canal behind 6207 Waverly Road.
980715	MNW9816					M	288	FL	Pinellas	Old Tampa Bay	27	82	St. Petersburg, washed ashore on Gandy Beach. S of Tampa
980711	MNW9817					M	254	FL	Manatee	Terra Ceia Bay	27	82	Terra Ceia, just south of Gus Point and red channel marker #10, on sandbar. S of Tampa
980714	MNW9819					M	241	FL	Hillsborough	Alafia River	27	82	Gibsonton, Williams Park, 6401 Riverview Drive. S of Tampa

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980715	MNW9820					F	210	FL	Citrus	Homosassa River	28	82	Homosassa, approx. 1/8 mile west of Magic Manatee Marina, along Halls River Road, approx. 50 yards from Marker #5, shallow water.
980715	MNW9821					F	250	FL	Manatee	Braden River	27	82	Bradenton, King Ranch, floating behind 5504 40th Avenue East. S of Tampa
980715	MNW9822					M	311	FL	Franklin	Ochlockonee River	29	84	Ochlockonee River State Park, approx. 1/4 mile east of McIntyre Landing between a derelict train tressel bridge & SR319 bridge.
981008	MNW9824					M	299	FL	Hernando	Gulf of Mexico	28	82	Bayport, on an oyster bar approximately 400 yards SW of the Bayport Park Fishing Pier.
980901	MNW9825					M	342	FL	Manatee	Tampa Bay	27	82	Terra Ceia, one-half way between Rattlesnake Key and the south base of the Skyway Bridge-approx. via phone from citizen. S of Tampa
981002	MNW9826					F	289	FL	Manatee	Sarasota Bay	27	82	Cortez, between Markers #47 & 49, on a mangrove island (bird sanctuary). S of Tampa
990202	MNW9903					F	237	FL	Gulf	St. Joseph Bay	29	85	Port St. Joe, 200 yards offshore on a sandbar just east of City Pier.
990305	MNW9904					F	228.5	FL	Citrus	Withlacoochee River	29	82	Yankeetown, north bank, approximately 1000 yards upstream of green marker #43.
990222	MNW9905					M	308	FL	Hillsborough	Little Manatee River	27	82	Ruskin, of the corner of a vacant lot at 1023 Canal Street. S of Tampa

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990222	MNW9906	▨	■	▩	▩	M	324	FL	Pinellas	Tampa Bay	27	82	St. Petersburg, along the shoreline approximately 50 yards south of the main channel to the Bartow Power Plant. S of Tampa
961014	MSE9625	▩	▨	□	▨	F	212	FL	Palm Beach	Lake Worth	26	80	Riviera Beach, approximately 1 mile north of the Lake Worth Inlet, west bank, residential canal behind 3640 Palm Drive.
961219	MSE9628	▩	▨	□	□	M	315	FL	Dade	Biscayne Bay	25	80	Homestead, floating in Biscayne Bay due east of the entrance to Mowry Canal.
961230	MSE9629	▩	▩	□	□	F	140	FL	Martin	Indian River	27	80	Seawall's Point, near channel marker # 235, west of the St. Lucie Inlet.
970226	MSE9704	▩	■	□	■	M	347	FL	Dade	Little River	25	80	Miami Shores, approximately 1/2 mile upstream of the mouth of Little River, behind 777 NE 77th Terrace.
970319	MSE9706	▩	■	□	□	F	351	FL	Dade	Atlantic Ocean	25	80	Tail: Fisher Island, Atlantic Ocean on east side of the island. Body: 1 mile offshore of North Miami Beach.
970321	MSE9707	▩	▨	□	□	M	309	FL	Dade	Government Cut	25	80	Miami Beach, approximately 1/2 mile west of the Atlantic Ocean entrance to Government Cut at the end of the USCG basin.
970321	MSE9708	▩	▨	□	▨	M	317	FL	Dade	Atlantic Ocean	25	80	North Miami Beach, approximately 1/2 mile north of Bakers Haulover Inlet on Haulover Beach County Park.
970410	MSE9710	▩	▩	□	□	F	202	FL	Martin	Willoughby Creek	27	80	Stuart, Indian River, in the river at the north fork of the creek.

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970415	**MSE9711**	▓	▒	□	▓	F	140	FL	Dade	Miami River	25	80	Miami Springs, upstream of SFWMD S-26 and directly behind 709 South Royal Poinciana Blvd.
970421	**MSE9712**	▓	░	□	■	F	214	FL	Dade	Biscayne Bay	25	80	Homestead, Biscayne National Park, at the mouth of Moody canal and downstream of SFWMD structure S-21A.
970428	**MSE9713**	▓	░	□	░	F	232	FL	Palm Beach	Lake Worth ICW	26	80	North Palm Beach, 700 yards east of Lake Worth on NE side of US 1 HWY bridge.
970529	**MSE9715**	▓	■	□	■	F	120	FL	Palm Beach	Lake Worth	26	80	Singer Island, Blue Heron Blvd. Causeway.
970724	**MSE9716**	▓	▒	□	░	M	304	FL	Dade	Miami River	25	80	Miami, Miami Canal upstream of SFWMD Structure S-26 and 150 yards east of NW 36th Street bridge.
970724	**MSE9717**	▓	▒	□	▓	F	285	FL	St. Lucie	Indian River	27	80	Ft. Pierce, on the west bank approximately 1.5 miles south of Ft. Pierce Inlet.
970724	**MSE9718**	▓	░	□	▒	F	276	FL	Martin	St. Lucie Canal	27	80	Tropical Farms, on the east bank of the canal, 50 yards downstream of the St. Lucie Lock and Dam COE structure S-80.
970825	**MSE9721**	▓	□	□	□	M	220	FL	Dade	Blue Lagoon Lake	25	80	Miami, on the north side of the lake off of the west end of the Miami Canal, directly behind the Hilton Hotel.
970826	**MSE9723**	▓	░	□	■	F	241	FL	Palm Beach	Jupiter Inlet	26	80	Jupiter, 100 yards south of the Jupiter Inlet just off the beach.
971113	**MSE9726**	▓	■	□	□	M	284	FL	Dade	Mowry Canal	25	80	Homestead, approximately 200 yards E of water control structure SFWD S-201.

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971217	MSE9727	■	■	□	□	M	262	FL	Dade	Black Creek Canal	25	80	Homestead, Black Creek Canal, Black Point Park Marina, downstream of SFWMD S-21 structure.
980102	MSE9729	■	▒	□	□	M	297	FL	Martin	Indian River	27	80	Stuart; Indian River East shoreline, 1000 yards South of Stuart (A1A) Causeway.
980102	MSE9730	■	■	□	□	M	307	FL	Martin	Peck Lake	27	80	Hobe Sound, ICW, North End of Peck Lake,; 300 feet East of channel marker 16 on shoreline.
	MSE 9772	■	▒	□	□								
980106	MSE9801	■	■	□	□	F	302.5	FL	Dade	Biscayne Bay	25	80	Homestead, approximately 1000 yards directly outside the mouth of North Canal.
980120	MSE9803	■	▒	□	□	M	255	FL	Broward	Intracoastal Waterway	26	80	Fort Lauderdale, one mile South of Port Everglades Inlet.
980120	MSE9804	■	▒	□	■	M	320	FL	Dade	Biscayne Bay	25	80	Miami, one-half mile South of Julie Tuttle Causeway on the West bank.
980120	MSE9805	■	■	□	▒	M	245	FL	Palm Beach	Lake Wyman	26	80	Boca Raton, just North of channel marker 57 on the East bank.
980204	MSE9806	■	▒	□	□	M	273	FL	Dade	Biscayne Bay	25	80	Homestead, 50 feet outside mouth of SFWMD S-20F, Mowry Canal.
980204	MSE9807	■	■	□	□	F	250	FL	Broward	Hillsboro Inlet	26	80	Lighthouse Point, Intracoastal waterway, 100 yards north of the Hillsboro Inlet.
980205	MSE9809	■	▒	□	□	M	270	FL	Dade	Little River	25	80	Miami, one half mile upstream of the mouth of the river.
980221	MSE9810	■	□	□	□	F	314	FL	Broward	Port Everglades discharge canal	26	80	Ft. Lauderdale; Port Everglades discharge canal mangrove lagoon.
980221	MSE9811	■	▒	□	▒	M	220	FL	Palm Beach	Atlantic Ocean	26	80	Jupiter, 50 yards north of the Jupiter Inlet on the beach.

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980228	MSE9812					F	280	FL	Broward	Port Everglades-ICW	26	80	Ft. Lauderdale, approximately three quarters of a mile South of Port Everglades Inlet on the East bank.
980324	MSE9814					F	202	FL	Martin	Manatee Pocket	27	80	Port Salerno; Manatee Pocket, at the end of Coquina Street.
980522	MSE9815					M	122	FL	Monroe	Tarpon Basin	25	80	Key Largo, in a residential canal behind West 1st Street. Miami-Dade county
980707	MSE9816					M	308	FL	Palm Beach	ICW	26	80	Boca Raton; Intercoastal Waterway in the Bel Marra area, behind 891 Appelby Street.
980707	MSE9817					M	270	FL	Monroe	Buttonwood Sound	25	80	Key Largo; Florida Bay, Buttonwood Sound: one quarter mile East of the ICW, between Grouper Cut and Baker Cut. Miami-Dade county
980708	MSE9818					M	121	FL	Broward	Dania Cut Off Canal	26	80	Dania, residential canal at the North end of SW 39th Way in Aqua Isle's Mobil Home Park.
980708	MSE9819					F	133	FL	Martin	North Fork Loxahatchee River	26	80	Hobe Sound, within Jonathon Dickinson State Park, approximately 2 miles North of Trapper Nelson's site.
980711	MSE9820					M	199	FL	Palm Beach	Lake Worth Creek	26	80	Juno Beach; ICW, 4.5 miles south of Jupiter Inlet and directly beneath the Donald Ross bridge.
980624	MSE9821					M	260	FL	Dade	Snake Creek Canal	25	80	Miami Gardens; residential canal off Snake Creek canal, 10 yards east of N.W. 181 St. and 78 Ave. bridge.
980714	MSE9822					F	370	FL	Dade	SFWMD C-111 Canal	25	80	Florida City, C-111 Canal, 3.7 miles north of SFWMD S-178.

Date	Field ID	FFWCC region	Assigned region	MPSWG region	Assigned region	Sex	Length	State	County	Waterway	Latitude degrees	Longitude degrees	Locality
980715	MSE9823	▓	▒	░	░	F	136	FL	Broward	Dania Cut-Off Canal	26	80	Dania, near the juncture of S. New River Canal in the FPL Broward County inland Plant intake canal.
980714	MSE9824	▓	▒	░	▓	F	335	FL	Martin	North Fork St. Lucie River	27	80	Palm City, North Fork of St. Lucie River half mile east of entrance of Bessey Creek, 76 SW Riverway Blvd. Seagate Harbor.
980720	MSE9825	▓	▓	░	░	F	265	FL	Dade	Bakers Haulover Inlet-ICW	25	80	North Miami Beach, 1 mile south of Bakers Haulover Inlet, near marker 14.
980730	MSE9826	▓	▓	░	░	F	110	FL	Martin	Loxahatchee River	26	80	Tequesta, Main Fork of the Loxahatchee River, at the South end of Jonathon Dickinson State Park.
990204	MSE9902	▓	█	░	█	F	220	FL	Broward	Port Everglades-ICW	26	80	Ft. Lauderdale, Port Everglades, 1/4 mile southwest of USCG Station, along seawall.
990223	MSE9904	▓	▓	░	░	M	212	FL	Dade	Biscayne Bay, Intracoastal Waterway	25	80	Miami Beach, approximately 1/2 mile south of 79th Street Causeway, east side of channel.
990223	MSE9905	▓	▒	░	░	F	295	FL	Palm Beach	Lake Worth Creek	26	80	Jupiter, app. 1/4 mile south of Indiantown Rd. (SR706) Bridge on west bank of ICW. Channel Marker 14.
971113	MSW9754	█	█	█	█	M	277	FL	Lee	Caloosahatchee River	26	81	Cape Coral, Piney Point, behind 5976 SW 1st Court.
971120	MSW9755	█	█	█	█	M	255	FL	Lee	San Carlos Bay	26	81	Ft. Myers Beach, Bunche Beach, grounded 300 yards S. of Bunche Beach Road.
971120	MSW9756	█	█	█	▒	F	251	FL	Lee	Caloosahatchee River	26	81	East Ft. Myers, behind 5203 Lake Caloosa Drive.
971109	MSW9759	█	▒	█	▒	M	243	FL	Lee	Caloosahatchee River	26	81	Fort Myers, Shell Point Village, in the lagoon behind Grandview

Date	Field ID	FFWCC region	Assigned region	MPSWG region	Assigned region	Sex	Length	State	County	Waterway	Latitude degrees	Longitude degrees	Locality
971125	MSW9765	■	░	■	■	M	254	FL	Charlotte	Ainger Creek	26	82	Englewood, floating 100 yards SW of the Placida Road Bridge.
971126	MSW9767	■	▓	■	■	M	209	FL	Collier	Gordon River	26	81	Naples, beached on a sandbar 50 yards behind Naples Marina at 475 North Road.
971126	MSW9771	■	■	■	■	M	255	FL	Lee	Mullock Creek	26	81	Island Park, Ten Mile Canal, floating along the southern edge of the barrow pit at Alico Road and US 41.
971202	MSW9774	■	▒	■	■	M	222	FL	Collier	Henderson Creek	26	81	Belle Meade, floating in the mangroves 1000 feet due north of the Rookery Bay NERR Docks.
971224	MSW9776	■	░	■	░	F	315	FL	Lee	Orange River	26	81	Ft. Myers, floating 500 feet NW of the Route 80 Bridge.
980106	MSW9779	■	▒	■	■	M	271	FL	Lee	Caloosahatchee River	26	81	Fort Myers, floating between red markers #26 & 28.
980107	MSW9781	■	▒	■	▓	F	170	FL	Lee	Caloosahatchee River	26	81	Ft. Myers, floating behind 517 Bayside Drive in a residential canal.
980107	MSW9782	■	▒	■	▓	F	221	FL	Lee	Caloosahatchee River	26	81	North Fort Myers, floating 50 feet offshore behind 111 East North Shore Avenue.
980203	MSW9802	■	▒	■	▓	M	212	FL	Lee	Caloosahatchee River	26	81	Ft. Myers, Shell Point Village, in the NE corner of the lagoon behind Grandview Road.
980316	MSW9805	■	■	■	▓	M	246	FL	Lee	Caloosahatchee River	26	81	Ft. Myers, Floating 1000 feet due south of Marsh Point.
980318	MSW9806	■	■	■	■	M	337	FL	Collier	Little Hickory Bay	26	81	Bonita Beach, floating behind 264 Lely Beach Road, 125 yards due east of Marker 14. Lee county
980420	MSW9808	■	■	■	▓	M	196	FL	Collier	Faka Union Canal	25	81	Port of the Islands, floating at the end of the most SE finger canal in the SE corner.

Date	Field ID	FFWCC region	Assigned region	MPSWG region	Assigned region	Sex	Length	State	County	Waterway	Latitude degrees	Longitude degrees	Locality
980311	MSW9809	■	▨	■	■	U	340	FL	Monroe	Roberts River	25	80	Flamingo; Roberts River approximately 3 miles up river of Whitewater Bay.
980421	MSW9810	■	▨	■	▨	M	101	FL	Lee	Imperial River	26	81	Bonita Springs, beached behind 3831 Riviera Circle.
980421	MSW9812	■	□	■	▨	F	117	FL	Lee	Estero Bay	26	81	Ft. Myers Beach, Black Island, Little Carlos Pass, in a finger canal due east of Lovers Key S.P. foot bridge.
980421	MSW9813	■	■	■	▨	M	118	FL	Lee	Gasparilla Sound	26	82	Boca Grande, floating behind 1640 Treasure Lane.
980421	MSW9814	■	▨	■	▨	M	136	FL	Lee	Matlacha Pass	26	82	Matlacha, Matlacha Isles, at the south end of NW 39th Avenue.
980421	MSW9815	■	■	■	▨	F	171	FL	Lee	Orange River	26	81	Ft. Myers, floating in the lagoon in the FPL Power Plant discharge canal.
980421	MSW9816	■	■	▨	▨	F	135	FL	Sarasota	Sarasota Bay	27	82	Nokomis, Shakett Creek, floating behind 605 Bonita Drive.
980421	MSW9817	■	■	■	▨	M	214	FL	Collier	Chokoloskee Bay	25	81	Everglades City, Lane Cove, 1 1/2 Nautical miles due east of the mouth of Barron River.
980421	MSW9818	■	□	■	□	M	130	FL	Collier	Collier Bay	25	81	Marco Island, floating in a residential canal behind 890 San Marco Drive.
980522	MSW9820	■	■	■	▨	M	252	FL	Charlotte	Lemon Bay	26	82	Grove City, Cedar Point, floating at the mouth of Ainger Creek, 1750 feet SW of 775 Bridge.
980522	MSW9821	■	▨	■	▨	M	206	FL	Collier	Chokoloskee Bay	25	81	Everglades City, grounded adjacent to 1180 Chokoloskee Drive.
980709	MSW9824	■	■	■	■	F	122	FL	Lee	Imperial River	26	81	Bonita Springs, floating across the river at the end of Del Lane.

Date	Field ID	FFWCC region	Assigned region	MPSWG region	Assigned region	Sex	Length	State	County	Waterway	Latitude degrees	Longitude degrees	Locality
980709	**MSW9825**	■	■	■	■	M	127	FL	Charlotte	Alligator Creek	26	82	Punta Gorda, Burnt Store Isles, floating in the perimeter canal across from 5060 San Massino Drive.
980709	**MSW9826**	■	■	■	■	M	97	FL	Charlotte	Gasparilla Sound	26	82	Placida, Coral Creek, swimming in a canal behind G row, Placida Mobile Home Park.
980709	**MSW9827**	■	■	■	▨	M	123	FL	Lee	Caloosahatchee River	26	81	North Ft. Myers, floating in a canal behind 50 Skyline Drive.
980709	**MSW9828**	■	■	■	▨	F	272	FL	Charlotte	Gasparilla Sound	26	82	Placida, floating 90 yards due north of green marker #3.
980619	**MSW9830**	■	▨	■	■	F	135	FL	Lee	Caloosahatchee River	26	81	Ft. Myers, Deep Lagoon, floating 20 yards SW of red marker #4.
980711	**MSW9831**	■	▨	■	■	F	222	FL	Lee	Calooshatchee River	26	81	North Ft. Myers, floating in a residential canal behind 1336 Harborview Drive.
980720	**MSW9832**	■	□	■	▨	M	117	FL	Charlotte	Peace River	26	82	Punta Gorda, in a culvert off the Northbound entrance ramp to I-75 from Rt. 17, off Broad Creek.
980713	**MSW9833**	■	▨	■	■	F	245	FL	Lee	Tarpon Bay	26	82	Sanibel, at the weir at the west end of Sanibel River, 480 yards due west of the end of Tarpon Bay Road.
980715	**MSW9834**	■	■	■	▨	M	204	FL	Charlotte	Lemon Bay	26	82	Placida, floating at the end of Panama Blvd., at the Ferry boat ramp.
980729	**MSW9836**	■	▨	■	■	F	298	FL	Glades	Lake Okeechobee	26	81	Lakeport, below Fisheating Bay in Monkey Box floating near cattails.
980716	**MSW9837**	■	□	■	▨	M	265	FL	Lee	Matanzas Pass	26	81	Ft. Myers, Hurricane Bay, floating in a residential canal behind 17740 Broadway Ave.

Date	Field ID	FFWCC region	Assigned region	MPSWG region	Assigned region	Sex	Length	State	County	Waterway	Latitude degrees	Longitude degrees	Locality
981002	**MSW9838**	■	▨	■	■	F	325	FL	Collier	Faka Union Canal	25	81	Port of the Islands, (approximate location: end of canal near Marker #52)
980805	**MSW9840**	■	■	■	▨	M	216	FL	Lee	Caloosahatchee River	26	81	Ft. Myers, west of Midpoint Bridge, 100 feet offshore of 10090 McGregor Blvd.
980805	**MSW9841**	■	▨	■	■	F	300	FL	Charlotte	Peace River	26	82	Port Charlotte, beached behind 24225 Harborview Road, between I-75 and US Hwy 41 bridges.
980904	**MSW9842**	■	■	■	■	F	310	FL	Monroe	Chokoloskee Bay	25	81	Chokoloskee, approximately 1 mile SE of Chokoloskee Island, within Chokoloskee Bay. Collier county
980904	**MSW9843**	■	░	■	░	F	161	FL	Charlotte	Peace River	26	81	Harbor Heights, floating in the river off the W. side of a small island, approx. 1/2 mile E. of I-75 Bridge.
980908	**MSW9844**	■	■	■	■	F	256	FL	Lee	Caloosahatchee River	26	81	North Ft. Myers, NE side of Edison Bridge, floating on rip-rap at foot of bridge.
981030	**MSW9845**	■	■	▨	■	F	276	FL	Sarasota	Myakka River	27	82	North Port, Harbor Cover Mobile Home Park, floating at the end of Harbor Cove canal and the intersection of the Myakka River.

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BIOGRAPHICAL SKETCH

Angelica Garcia-Rodriguez is a biological technician with the U.S. Geological Survey's Florida Caribbean Science Center-Biological Resources Division, where she works on the Sirenia project. A biologist with seven years of experience applying population genetics to the conservation and management of endangered species, she received her B.S. degree in 1987 at the Universidad Central de Venezuela and her M.S. degree in 1995 in marine biology at Scripps Institute of Oceanography, University of California, San Diego.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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